Ch 17
(305/1961)

UDC 661.728.221.6:547.992.3:543.8

ACTA POLYTECHNICA SCANDINAVICA

CHEMISTRY INCLUDING METALLURGY SERIES No. 17

KAJ FORSS

The Composition of a Spent Spruce Sulfite Liquor

Finnish Contribution No. 26

Also published as Communication No. 263 of The Finnish Pulp and Paper Research Institute

Helsinki 1961

ACTA POLYTECHNICA SCANDINAVICA

. . . a Scandinavian contribution to international engineering sciences

Published under the auspices of the Scandinavian Council for Applied Research

in *Denmark* by the Danish Academy of Technical Sciences

in *Finland* by the Finnish Academy of Technical Sciences, the Swedish Academy of Engineering Sciences in Finland, and the State Institute for Technical Research

in *Norway* by the Norwegian Academy of Technical Science and the Royal Norwegian Council for Scientific and Industrial Research

in *Sweden* by the Royal Swedish Academy of Engineering Sciences, the Swedish Natural Science Research Council, and the Swedish Technical Research Council

Acta Polytechnica Scandinavica consists of the following sub-series:

Chemistry including Metallurgy Series, Ch
Civil Engineering and Building Construction Series, Ci
Electrical Engineering Series, El
Mathematics and Computing Machinery Series, Ma
Mechanical Engineering Series, Me
Physics including Nucleonics Series, Ph

For subscription to the complete series or to one or more of the sub-series and for purchase of single copies, please write to

ACTA POLYTECHNICA SCANDINAVICA PUBLISHING OFFICE

Box 5073
Stockholm 5 Phone 67 09 10

This issue is published by
THE FINNISH ACADEMY OF TECHNICAL SCIENCES
Helsinki, Finland

UDC 661.728.221.6:547.992.3:543.8

THE COMPOSITION OF A SPENT SPRUCE SULFITE LIQUOR

by

KAJ FORSS

Thesis for the degree of Doctor of Philosophy
accepted by the Abo Akademi.

ACTA POLYTECHNICA SCANDINAVICA
Chemistry Including Metallurgy Series Ph 17
(AP 305/1961)

Borgå 1961
Tryckeri- & Tidnings Ab.

PREFACE

This investigation was carried out at The Finnish Pulp and Paper Research Institute, Helsingfors, during the years 1956—1960. I am indebted to the Board of Directors of the Institute for the opportunity of performing the experimental work.

To Professor W. Jensen, D. Tech., the Managing Director of the Institute, I wish to express my sincere gratitude for suggesting the subject of the study and for his kind help and encouragement during the investigation.

I am very grateful to Professor H. Aspelund, D. Tech., Åbo Akademi, and Mr. Gust.-Ad. Holmberg, Ph. D., Åbo Akademi, for their advice on various occasions.

To my friend and co-worker, Mr. K.-E. Fremer, Ph. M., I wish to tender my thanks for proficient help with the experimental work.

I also wish to express my thanks to the staff of the Institute. I mention particularly Mr. B. Anthoni, Ph. D., Mrs. L. Blomberg, Ph. M., Mr. B. C. Fogelberg, Ph. M., Miss A. Grönvik, M. Sc., Mr. S. K. Kahila, Ph. Lic., and Mr. K. Passinen, Lic. Tech.

I am very much obliged to Mr. J. J. Lindberg, Ph. D., University of Helsinki, for discussions and suggestions concerning the interpretation of the infrared spectra.

To Mr. E. R. Korte, Ph. M., I wish to tender my thanks for the translation of this dissertation into English and for valuable discussions.

Finally I extend my thanks to my wife for her help in the preparation of the manuscript and proofreading and for all her encouragement.

Helsingfors, Finland, April, 1961.

K. F.

This issue has been printed with the aid of a grant from the Finnish Academy of Technical Sciences, to which I wish to express my sincere gratitude.

Helsingfors, Finland, November, 1961.

K. F.

CONTENTS

INTRODUCTION

The production of sulfite pulp in Finland during 1960 was approximately 1,300,000 metric tons. As nearly equal parts of the original wood are distributed between the spent sulfite liquor and the pulp, the more than one million tons of dry matter in the spent liquor represents a potential source of raw material and an economic problem of major importance.

Despite all the efforts that have been made during almost one hundred years to utilize the compounds of spent sulfite liquor, the results have been limited to the production of relatively small quantities of alcohol, yeast, torula yeast, vanillin, dispersing agents and some other products. The main reason why very little commercial use has been made of spent sulfite liquor is that our knowledge of the composition of the liquor and the structure of its constituents has been too meagre. A further reason is that sufficiently selective methods have not been devised for isolating the various compounds.

Of the constituents of spent sulfite liquors, only the monosaccharides have been studied systematically in respect of their formation and occurrence. The views about the nature of the incompletely hydrolyzed hemicellulose compounds are widely divergent. Thus, for example, Casey [1] has reported that the dry matter in spent sulfite liquor contains 15—20 per cent monosaccharides and 10—15 per cent incompletely hydrolyzed carbohydrates. The corresponding percentages found by Nikitin [2] are 12—33 per cent and 0.1—11 per cent. According to Hägglund [3] again, spent sulfite liquor generally contains only monosaccharides.

Whereas polysaccharides represent a definite category of compounds, at least by definition, it is not easy to determine what is understood by lignosulfonic acids. It is obvious that the lignosulfonic acids are polydisperse but it is unknown to what extent and how the molecular weights of the acids vary with the cooking conditions. Also the compositions and properties of the lignosulfonic acids seem to vary. The acids are hence conventionally divided into alpha- and beta-lignosulfonic acids. It is obviously due to the difficulty of defining the lignosulfonic acids that Nikitin reported that the dry matter of spent sulfite liquor contains 23—61 per cent lignosulfonic acids and 2—43 per cent tannins, whereas

other investigators such as Casey do not mention the occurrence of tannins but state that the lignosulfonic acid content is about 60 per cent.

When the author undertook in 1956 to carry out an investigation of spent sulfite liquor, it became obvious already at an early stage that the first main objective would have to be the isolation in a pure state of the constituents, especially the lignosulfonic acids, of spent sulfite liquor. As it did not seem possible to define unambiguously a pure lignin preparation, the decision was made to embark on the somewhat laborious task of determining which substances are or are not present as impurities in the fractionated lignosulfonic acids and of comparing different lignosulfonic acid fractions with each other. For this reason the work described below comprised both an extensive review of the literature and experimental investigations that dealt with the varied reaction products of the sulfite cook. Owing to the wide scope of the study, it was not possible to attempt a complete clarification of the constituents of a spent sulfite liquor, but only to obtain a general picture of their nature. It should perhaps be stressed in this connection that the results reported strictly relate only to a spent sulfite liquor from an industrial cook of spruce wood (*Picea excelsa*) that yielded a strong pulp.

THE STRUCTURE AND CHEMICAL COMPOSITION OF SPRUCE WOOD

THE ANATOMY OF SPRUCE WOOD

Spruce wood has a relatively simple structure (Fig. 1) for 93 to 95 per cent of the wood consists of tracheids or wood cells. These cells die during the lignification process and then no longer contain living plasma. A high proportion (95—97 %) of the tracheids run parallel to the trunk of the tree. These cells are up to several millimeters long but their thickness is only about 30 microns. The tracheids of spring wood, which have thin walls, mainly serve as channels for the passage of water from the root to the crown. They are connected with each other through bordered pits. The tracheids of the summer wood, which are thick-walled and function as weight-bearing elements, are, on the other hand, connected through slit-like pits.

In addition to these longitudinal tracheids, spruce wood contains a low proportion of radial tracheids which occur in one or a few rows above and below horizontal wood rays formed by living parenchymatous cells. The radial tracheids allow the passage of water in the horizontal direction and are connected with the longitudinal tracheids through bordered pits and with the parenchymatous cells through semi-bordered pits.

Also resin canals are found in spruce wood. These are tubular and run both vertically and radially. They vary from 10 to 80 cm in length. The vertical canals are approximately 0.08 mm thick and the radial canals approximately 0.03 mm thick. They are surrounded by thick-walled parenchymatous cells, epithelial cells, which excrete the resin into the resin canals.[4]

According to Trendelenburg [5] spruce wood has the following composition (by volume):

Tracheids	93 — 95	per cent
Wood rays	5 — 7	per cent
Parenchymatous cells	—	per cent
Resin canals	0.2 — 0.3	per cent

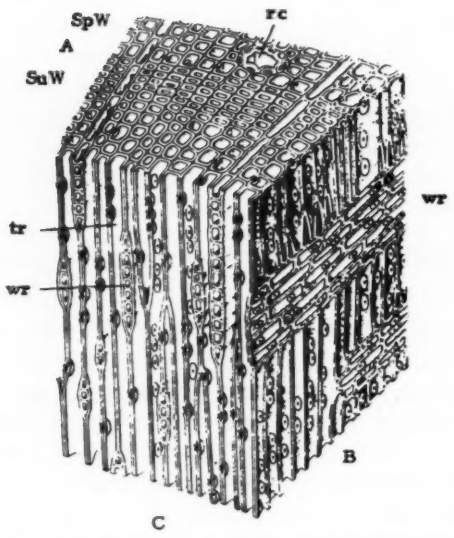


Fig. 1. The structure of spruce wood according to Schmeil-Seybold.[237] A transverse section, B radial section, C tangential section, SpW spring wood, SuW summer wood, tr tracheids, wr wood rays, rc resin canals.

(Reprinted by permission of the author and Quelle & Meyer Verlag, Heidelberg)

Trendelenburg thus does not give a percentage for the parenchymatous cells, i. e. the wood ray parenchyma and the epithelial cells, which together with the wood ray tracheids compose the 0-fiber fraction in pulp manufacture.

THE STRUCTURE OF THE TRACHEIDS

According to the views of Kerr and Bailey [6] as modified by Meier,[7] the tracheids have the structure shown in Fig. 2.

The Middle Lamella

The middle lamella is formed by substance lying between the tracheid cells. The compound middle lamella comprises the middle lamella and the primary walls on both sides of the latter. This latter designation has been introduced because there are no definite phase boundaries between the middle lamella and the primary walls. According to Mühlethaler,[9]

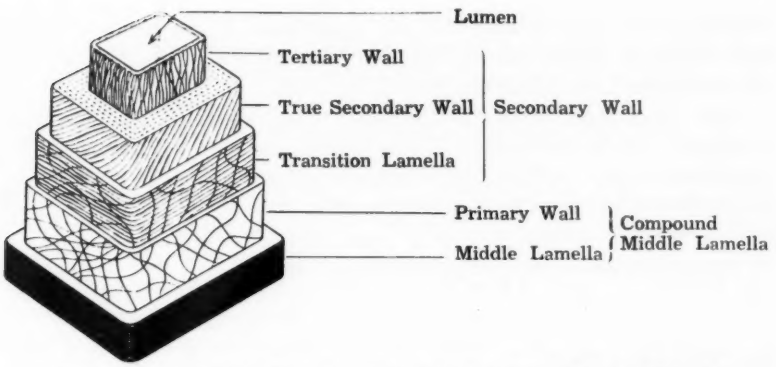


Fig. 2. **The wall structure of a conifer tracheid according to Meier.[8]**
(Reprinted by permission of the author and Springer-Verlag, Berlin, Göttingen, Heidelberg)

there exist microfibrils that extend from one primary wall through the middle lamella to the primary wall of the adjoining cell.

The thickness of the compound middle lamella varies from a few tenths of a micron to about two microns depending on the tree species and the degree of lignification.[10]

In young cells the middle lamella consists primarily of pectin but after cellulose appears in the cell walls the middle lamella begins to lignify.[11] Bailey[12] found the middle lamella of Douglas fir (Pseudotsuga taxifolia) to contain approximately 72 per cent lignin and 14 per cent pentosans. According to Lange[13] the lignin content of the compound middle lamella in spruce varies from 60 to 90 per cent.

The Primary Wall

The primary wall is formed by a very coarse network of carbohydrate microfibrils which is highly incrusted with lignin. The thickness of the primary wall of a spruce tracheid does not exceed 0.1 micron.[7] One of the most characteristic properties of the primary wall is its high resistance to the action of cooking and bleaching agents. Cuprammonium solution is able to dissolve the primary wall only very slowly.[14] Besides this difference between the carbohydrates of the primary wall and the cellulose of the secondary wall, it may be noted that according to Meier[7] the carbohydrates of the primary and tertiary walls are exceptionally resistant to enzymatic degradation by wood-rotting fungi. Meier has therefore doubted whether the carbohydrate of the primary wall is identical with the cellulose of the secondary wall.

The degree of crystallization of the microfibrils of the primary wall is also definitely lower (34—37 %) than the degree of crystallization of the microfibrils of native cellulose (50—83 %).[15]

The high stability of the primary wall substance has awakened interest in the extent to which it is broken down in industrial cooking processes. Jayme and Hunger [16] have studied this question in the case of spruce sulfite and sulfate pulps. In many cases the primary wall could still be readily identified, but in some cases it had been transformed or completely destroyed in the pulping process.

The Secondary Wall

In the secondary wall, which is about 5 microns thick, it is possible to distinguish three layers which differ in their physical and chemical properties. These layers are the transition lamella, the true secondary wall and the tertiary wall.

Transition Lamella. Meier [7] called the outer layer of the secondary wall the transition lamella because its microfibrillar structure represents a structure intermediate between that of the primary wall and that of the secondary wall. Most of the microfibrils in the transition lamella run more or less transversely relative to the fiber axis but the outer layer contains also randomly oriented microfibrils. Meier reported the thickness of the transition lamella in delignified wood to be about 0.2 micron. According to Asunmaa and Lange [17] the cellulose content of the cell wall decreases from the lumen to the primary wall. As the transition lamella is more densely packed than the secondary wall, Meier [7] concluded that the microfibrils of the transition lamella are not pure cellulose.

True Secondary Wall. The greater part of the cellulose in the tracheid is located in tightly packed structures in the central layer which varies from one to five microns in thickness. The spruce fibrils have a spiral texture with an angle of 18 degrees between the fiber axis and the fibril direction. In spruce tracheids the fibrils are to a great extent embedded in lignin.[7]

Tertiary Wall. Like the other wall layers the tertiary wall is composed of both crystalline and amorphous substances. The crystalline components are microfibrils which according to Meier [7] run more or less parallel to the fiber axis. Meier and Yllner [18] state that the tertiary walls in spruce tracheids are probably partly composed of xylans. The

insolubility of the tertiary wall in cupriethylenediamine solution points to a low cellulose content.[19] This is further supported by the strong resistance exhibited by the tertiary wall to enzymatic degradation by fungi, in which respect it resembles the primary wall.[7]

When he studied the effect of sulfite and sulfate cooking liquors on the tertiary wall, Meier [20] found that the tertiary wall is only slightly affected by sulfate cooking liquor and offers a great resistance to the diffusion of the cooking liquor and the cellular components dissolved in it. The tertiary wall forms a barrier against the lumen that is approximately as impervious as that formed by the primary wall and the transition lamella against the middle lamella.

In the sulfate cooking process lignin and hemicellulose are dissolved equally readily through both barriers. The residual lignin and hemicellulose are evenly distributed throughout the cell wall.

In sulfite cooking the processes are quite different. The tertiary wall is degraded to a great extent and offers a much lower resistance to diffusion than the primary wall and the transition lamella. The cooking liquor therefore dissolves lignin and hemicellulose from the secondary wall primarily by way of the cell lumen. The extraction is also more complete than in sulfate cooking. The residual lignin and hemicellulose are then found mainly in the outer layers of the cell wall.

THE FORMATION OF THE TRACHEIDS

A tree grows in height in a zone called the apical meristem at the tip of the trunk and the ends of the branches. In this process so-called primary wood is formed which surrounds the pith. The increase in the thickness of the wood, the formation of the secondary wood, results from cell division in the cambium, a thin layer of cells between the bark and the wood. This cell layer forms new cells by division in the tangential direction. These new cells lie partly against the bark where they form the inner bark and partly against the wood. The tracheids lying against the wood have only a thin wall, the primary wall, and are separated from each other by the middle lamella which seems to be mainly composed of pectin. During and after the cell division the primary wall expands and when this expansion comes to an end the transition lamella is deposited and after this the true secondary wall and the tertiary wall. The first signs of lignin formation are noted at the cell corners in the region of the primary wall when the secondary wall begins

to form. After this the middle lamella is lignified, first in the tangential and then in the radial direction. The transition lamella, the true secondary wall and the tertiary wall are subsequently lignified in this order, but only after the cellulose skeleton has fully developed.[21, 11]

THE CHEMICAL COMPOSITION OF SPRUCE WOOD

It is difficult to obtain a reliable picture of the chemical composition of wood. In the first place, most of the cell wall constituents are macromolecular compounds which penetrate, and are possibly chemically bound to, each other. It is therefore frequently difficult to isolate these compounds without degrading them. In the second place, the composition of the wood of even the same species varies depending on the age and site of growth of the tree. The following analytical results of Gustafsson [22] give an idea of the quantitative distribution of the various main constituents of spruce wood, but do not reveal how these constituents are bound to one another in the wood (Table I).

Table I

The Composition of Dry Spruce Wood (*Picea excelsa*) According to Gustafsson [22]

Cellulose	glucan	42.8 % by weight
Hemicellulose	glucan*	
	mannan	10.8 % —»—
	xylan	5.5 % —»—
	galactan	3.9 % —»—
	araban	1.2 % —»—
	uronic acid units	2.8 % —»—
	acetyl and formyl groups	1.9 % —»—
Lignin		28.6 % —»—
Ether and alcohol solubles		1.5 % —»—
Ash and protein		1.0 % —»—
		100.0 % by weight

* According to Hägglund [23] hemicellulose contains 2 per cent glucan that is hydrolyzed with difficulty and 0.8 per cent that is readily hydrolyzed.

It may be briefly stated that the lignin content varies between 60 and 90 per cent in the compound middle lamella but decreases to less than 10—20 per cent in the vicinity of the cell lumen. The hemicellulose distribution is similar, for hemicellulose amounts to more than 60 per cent of the carbohydrates of the outer layer, but to less than 15 per cent of the carbohydrates in the layers adjoining the lumen. On the other hand, cellulose is the predominating component in the layer adjoining the lumen, but is present in only small amounts in the outermost layer.

THE REACTIONS OF SPRUCE WOOD COMPONENTS IN SULFITE COOKING

THE SULFITE PROCESS

In the sulfite pulping process wood chips are heated under pressure in an aqueous solution containing mainly bisulfite, hydrogen and calcium ions, but sodium, magnesium or ammonium ions may replace the calcium ions. In addition, the cooking liquor contains sulfurous acid and dissolved sulfur dioxide. The liquor is acid in reaction; at the beginning of the cook the pH is about 2, but it later decreases to about 1 owing to the formation of strong sulfonic acid groups.

In the first stage of the sulfite cook, the cooking liquor penetrates the chips at a low temperature. The wood swells considerably in the tangential and radial directions but insignificantly in the longitudinal direction. The cooking liquor enters the lumen at this stage and diffuses into the cell walls where it comes into contact with the lignin and carbohydrates. This impregnation of the chips takes place about one hundred times as rapidly in the longitudinal than in the transverse direction. The liquor passes from one tracheid to the other by way of the bordered pits and from the tracheids to the wood rays through the semi-bordered pits.

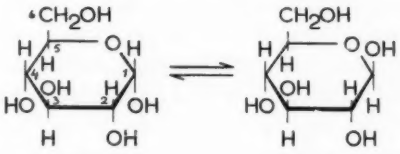
After the chips have become completely impregnated and the temperature has risen to 50—60° C, chemical reactions set in. If the temperature is allowed to rise too early during the impregnation process, a »burnt» cook may result since sulfur dioxide penetrates much more rapidly than the base and reacts with lignin at temperatures above 100 C° to form dark-colored condensation products.

The final cook takes place at a temperature not exceeding 130—150° C. The duration of the cook varies from about 6 to 14 hours depending on the maximal cooking temperature, the composition of the cooking liquor, the quality of pulp desired and the species of wood.[24]

CELLULOSE

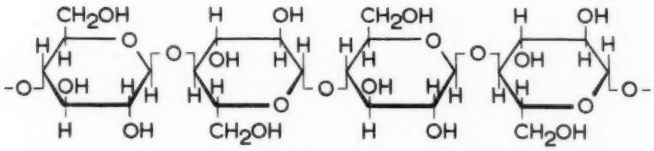
THE STRUCTURE OF CELLULOSE

Cellulose is a linear chain polymer composed of D-glucose units **(1)** bound by (1→4)-β-glucosidic linkages **(2)**.



α-D-Glucose β-D-glucose

(1)



Cellulose **(2)**

The molecular size of native cellulose is not known with certainty. According to Marx and Schulz [73] the degree of polymerization of fiber cellulose varies between 6500 and 8000, but carefully isolated cellulose from spruce has a degree of polymerization of 3300, whereas the degree of polymerization of industrial spruce cellulose is 1400.

The cellulose molecules form biological units called microfibrils which are visible in an electron microscope. These microfibrils have a diameter of about 250 Å but are of variable length. There are regions in the microfibrils which have a crystalline structure. These regions, which are known as micelles, are separated from each other by paracrystalline phases.

According to Frey-Wyssling [25] the microfibrils are not the smallest building stones but consist of ribbonlike elementary fibrils with a cross-section of 30 · 100 $Å^2$ or 50 · 60 $Å^2$. The elementary fibrils are composed of a crystalline chain lattice of cellulose and are surrounded by a layer of paracrystalline cellulose. This sheath which forms the intermicellar substance in the microfibrils is about 10 Å thick. As small molecules such as water are able to penetrate between the paracrystalline chains, the microfibrils swell readily. In the transverse direction the

microfibrils may be united in lamellae. Capillaries of the order of 100 Å in diameter are seen between the microfibrils, and these interfibrillar spaces may be filled by colloidal incrustations, lignin, etc.

THE REACTIONS OF CELLULOSE

Cellulose is only insignificantly altered during the sulfite cooking process, for Sundman [26] found no glucose in the spent liquor from a sulfite cook giving a strong pulp. The spent sulfite liquor from a rayon pulp cook carried out at a low pH with a high final temperature contained glucose in an amount corresponding to 5—15 per cent of the hexose fraction. It is impossible to say to what extent this glucose is derived from cellulose or from difficultly hydrolyzed hemicellulose components such as glucomannans.

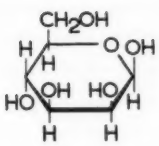
HEMICELLULOSE

THE CONSTITUENTS OF HEMICELLULOSE

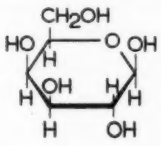
The polysaccharides that are closely associated with cellulose in the cell wall are called hemicellulose. The difference between hemicellulose and cellulose and other constituents of wood such as pectins, starch and polyuronic acids is, however, vague owing to experimental difficulties and to indefinite nomenclature. The simplest of these molecules have short straight chains but most of the molecules have side chains. In many cases these side chains are assumed to be evenly distributed along the main chain. The degree of polymerization seems to vary between 50 and 300 in carefully isolated hemicellulose components.[27]

Very little is yet known about the supermolecular structure of hemicellulose. There seem to exist both amorphous and highly crystalline as well as fibrillar and structureless hemicellulose components.

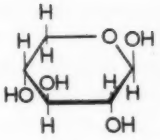
The question of the sugars that occur in hemicellulose was not solved before the late 1940's when Sundman, Saarnio and Gustafsson [28] succeeded in demonstrating that spruce wood contains only five monosaccharides, viz., the hexoses D-glucose **(1)**, D-mannose **(3)** and D-galactose **(4)** and the pentoses D-xylose **(5)** and L-arabinose **(6)**. In addition, spruce wood contains glucuronic acid **(7)**, 4-O-methyl-D-glucuronic acid **(8)**, and galacturonic acid **(9)**.[29]



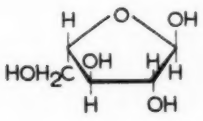
β-D-Mannose (3)



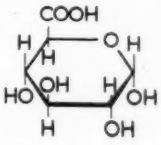
β-D-Galactose (4)



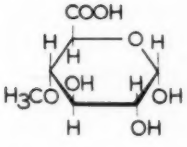
β-D-Xylose (5)



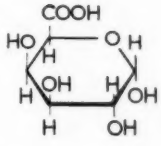
α-L-Arabinose (6)



α-D-Glucuronic acid (7)



4-O-Methyl-α-D-glucuronic acid (8)



α-D-galacturonic acid (9)

The wood contains acetic acid and possibly also formic acid esterified with xylans or other hemicellulose components.[22]

Gustafsson, Sundman, Pettersson and Lindh [30] have determined the amounts of monosaccharides in the hydrolyzates of spruce wood (Table II).

Table II

Carbohydrates in Spruce Wood (*Picea excelsa*) Hydrolyzates According to Gustafsson et al.[30]

Glucan	65.5 %
Mannan	16.0 %
Galactan	6.0 %
Xylan	9.0 %
Araban	3.5 %

The greater part of the glucan is derived from the cellulose. The mannan content is generally almost as high as the total content of the

next three carbohydrates. For comparison, it may be mentioned that the xylan content of birch wood (*Betula verrucosa*) is 39.0 per cent.

According to Gustafsson [22] the content of acetyl and formyl groups in spruce wood is 1.9 per cent.

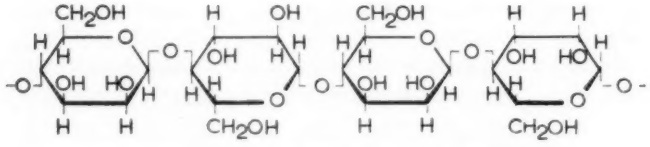
Jayme and Hahn [29] studied the uronic acids in various tree species. They found that spruce wood contains 1.7 per cent glucuronic acid and 1.6 per cent 4-O-methyl-D-glucuronic acid bound to the hemicelluloses. They found also 0.5 per cent galacturonic acid present as pectin together with lignin in the middle lamella.

The methoxyl groups occur in spruce wood mainly in the lignin and in the above-mentioned uronic acid. Hägglund and Sandelin [31] have found the methoxyl content of spruce wood to be 4.60 per cent, of which 4.04 per cent is in the lignin and 0.56 per cent in the carbohydrate fraction.

Our knowledge of the chemical structures of the various hemicelluloses is yet very limited.

Glucomannans

It was previously believed that the mannose in wood is present solely as mannans, i.e., mannose units united by (1→4)-glucosidic bonds, but in recent years Lindberg and Meier [32] and others have isolated pure glucomannans from wood in which the ratio of mannose to glucose units varies from 4:1 to 3:1. These glucomannans are evidently polymers joined by (1→4)-β-bonds (**10**).[33] The degrees of polymerization of glucomannans vary between 70 and 140. [32]



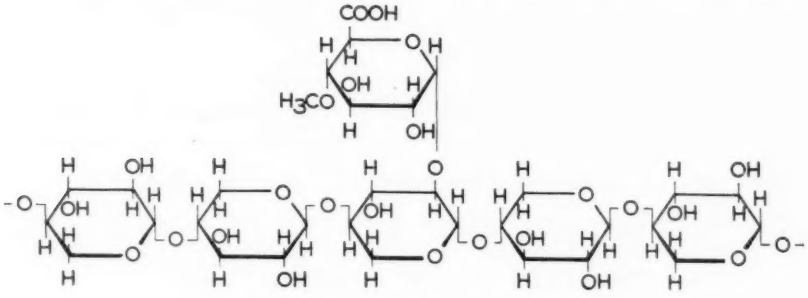
A section of a glucomannan chain (**10**)

According to Lindberg [34] a part of the glucomannans can be readily extracted from the wood, whereas the rest is extracted only with difficulty. The marked difference in extractability is probably due to the shape of the molecules within the wood and their location within the cell walls. Lindberg concluded further that the major part of the mannose residues in the hemicellulose is present in glucomannans.

In addition to glucomannans, spruce wood possibly contains also galactomannans or galactoglucomannans.[35]

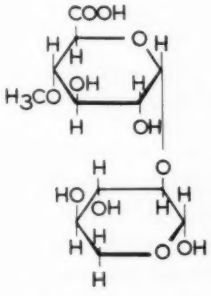
Xylans

Like the xylans isolated from other plants, the xylans of spruce wood are composed of chains of (1→4)-linked β-D-xylopyranose residues. By alkali extraction of chlorite holocellulose from spruce (*Picea excelsa*), Aspinall and Carter [36] were able to extract a xylan which like other wood xylans contained 4-O-methyl-D-glucuronic acid bound to the xylose units at the position 2 **(11)**. In the carbohydrate isolated by Aspinall and Carter, every fifth xylose unit had this uronic acid as a substituent.



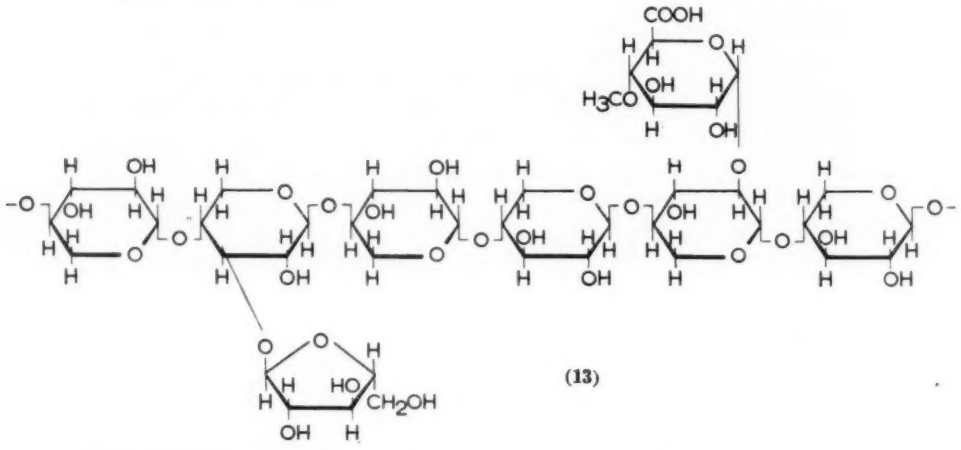
A section of a xylan chain **(11)**

As the bond between the uronic acid and the xylan chain is much more resistant to acid hydrolysis than a glucosidic bond, the aldobiuronic acid O-(4-O-methyl-α-D-glucosyluronic acid)-(1→2)-D-xylose **(12)** is usually obtained in the stepwise hydrolysis of xylans.[37]



O-(4-O-methyl-α-D-glucosyluronic acid)-(1→2)-D-xylose **(12)**

Xylans may also contain L-arabinofuranose side chains. Thus, for example, Saarnio [38] isolated by alkali extraction from spruce wood an araboglucuronoxylan with the following assumed repeating unit **(13)**:



(13)

This xylan was obtained in a yield of 2 per cent on the wood.

THE REACTIONS OF HEMICELLULOSE

The Hydrolysis of Hemicellulose

In the acid conditions prevailing in the sulfite digestion the long carbohydrate chains are gradually broken down to small fragments. The rate of the breakdown is determined partly by the susceptibility of the chemical bonds joining the structural units of the hemicellulose to hydrolysis and partly by the access of the digestion liquor to the various carbohydrates. The solubilities of the molecules in water increase inversely as their chain lengths and when the fragments become short enough they dissolve in the digestion liquor.

Monosaccharides in Spent Sulfite Liquor

In his study of the liberation of simple sugars in the sulfite digestion of spruce wood, Sundman [39] obtained the following results for the five monosaccharides which dissolve in the cooking liquor.

Arabinose. Arabinose is the first monosaccharide to dissolve in the sulfite digestion; this sugar can be detected already before the temperature has risen to 100°C. The arabinose content of the cooking liquor soon reaches a maximum and then decreases as the digestion continues until it finally disappears completely.

Xylose. Like arabinose xylose is relatively easily dissolved. In the digestion of conifers the xylose can be detected when the temperature has risen to 100°C. The amount of dissolved xylose increases rapidly at first, but it seems that its content does not decrease like that of arabinose toward the end of the digestion.

Galactose. As Hägglund and Klingstedt [40] had already observed, the galactans of coniferous wood are relatively easily hydrolyzed. Accordingly, galactose appears relatively early in the cooking liquor

Table III

Contents of Monosaccharides (g/l) in Spent Sulfite Liquors from Two Digestions of Spruce Wood (*Picea excelsa*) According to Gustafsson.[41]

		Liquor I	Liquor II
Digestion	Total SO_2	5.1 %	5.2 %
	CaO	1.0 %	0.7 %
	Maximum temperature	125°C	150°C
	Total cooking time	9.7 h	10 h
	Roe number of the pulp	9.7	2.1
Liquor	Galactose	2 g/l	3 g/l
	Glucose	1 g/l	5 g/l
	Mannose	13 g/l	18 g/l
	Arabinose	1 g/l	1 g/l
	Xylose	5 g/l	6 g/l

and its amount rises to a maximum already in the first stages of the cook.

Mannose. Mannans are definitely more resistant to the hydrolytic action of the digestion liquor than the polysaccharides that yield the monosaccharides just mentioned. The solution of mannose begins only after arabinose and galactose have been practically completely dissolved and continues to the end of the digestion.

Glucose. Glucose appears in the liquor toward the end of the

digestion. Its content increases with the length of the digestion period and with temperature. Under the conditions prevailing during a rayon cook, glucose is dissolved only after the temperature has exceeded 140°C. This does not mean, however, that this temperature is the lower limit for the dissolution of glucose, for glucose is liberated into the liquor during a longer period of digestion at 130°C.

The contents of the five monosaccharides in the liquors at the end of two sulfite digestions of spruce wood are shown in Table III.

Polysaccharides in Spent Sulfite Liquor

Roschier et al.[42, 43] observed that metal hydroxides such as those of copper (II), lead and antimony precipitate polysaccharides from alkaline solutions. Roschier and Eskola [44] employed this precipitation method to isolate the polysaccharides from the liquors of three sulfite digestions of spruce wood performed on a laboratory scale. Spruce chips were in all cases digested together with the same sodium bisulfite cooking liquor (total SO_2 : 7.35 %; combined SO_2 : 2.7 %) at the same temperature (150°C), but the duration of the cook was varied (0.5, 2 and 4 hours). The pulp produced in the first digestion was very hard and consisted mainly of sticks, whereas the third batch was cooked too long. The authors found that the liquor from the first digestion contained tetra-, tri- and disaccharides of both the hexosan and pentosan types. Large amounts of mannose, glucose and galactose and some xylose were liberated by hydrolysis. The liquor from the second digestion contained polysaccharides composed of mannose and glucose, whereas that from the third digestion contained only small amounts of polysaccharides that yielded mannose on hydrolysis.

Shaw also studied the polysaccharides present in sulfite liquors.[45] By dialysis, precipitation with alcohol, ion exchange and paper chromatography, he isolated from a spent sulfite liquor five polysaccharide fractions which together amounted to 3 — 7 per cent of the dry matter in the liquor. The degree of polymerization was found to vary from two to seven, but none of the fractions was found to be chromatographically pure. Hydrolysis showed that in these cases mannose was the dominating component. Glucose, xylose, galactose, and from one fraction rhamnose were isolated in addition to mannose. The author did not, however, give any information about the type of wood investigated or the cooking conditions.

Formation of Acetic and Formic Acids

As already mentioned (p. 19), the acetyl and formyl groups in wood are esterified with the carbohydrates of the hemicellulose. According to Hägglund [46] a large proportion of the acetyl groups are liberated at an early stage of the cooking process. The amounts of acetic and formic acids produced are as high as 25 g/kg wood.

It was previously believed that spruce wood contains about 0.3 per cent formyl groups,[47] but Timell [48] later showed that a part of the formyl groups result from the decomposition of the polysaccharides in the wood in acid and alkaline conditions. He concluded that if formyl groups occur in wood their amount does not exceed 0.01 — 0.02 per cent.

In analogy with the oxidation of aldoses to aldonic acids (p. 27), Stockman [49] showed that the aldehyde formic acid is oxidized to carbon dioxide and water during the sulfite digestion with the simultaneous formation of thiosulfate.

$$2\,HSO_3^- + 2\,HCOOH \rightarrow S_2O_3^{2-} + 2\,CO_2 + 3\,H_2O$$

The studies of Ahlén and Samuelson [50] revealed further that the formic acid in spent sulfite liquor amounts to only about 0.04 g/l, which is only one-tenth of the amount previously reported.

Formation of Methyl Alcohol

The methyl alcohol found in spent sulfite liquor is probably derived from the O-methyl-substituted carbohydrates. According to Bergström,[51] 8—10 kg of methyl alcohol is obtained for each ton of easily bleached pulp. This corresponds to about 4 kg per ton of dry wood. The greater part of the alcohol remains in the spent liquor, for only about 3 kg of the alcohol per ton of pulp is removed along with the relief gases.

Hemicellulose Components in the Pulp

The following analytical data for spruce sulfite pulps with a Roe number of 5 [22] give an idea of the carbohydrate composition of pulp (Table IV). The data reveal that large amounts of mannan, xylan, uronic acids, and acetyl and formyl groups are present in the pulp, whereas galactan and araban are absent.

Table IV

Analytical Data for Spruce Sulfite Pulps According to Gustafsson [22]

The data relate to pulps of Roe number 5. The values in brackets express the amounts of carbohydrates as percentages of the carbohydrates in the original wood.

Data		Pulp 1	Pulp 2	Pulp 3	Pulp 4
SO_2, total	%	6	6	9	9
Temperature, max.	°C	130	145	130	145
Time at max. temp.	h	4.2	1.4	2.5	0.6
Yield, total	%	50.7	49.3	51.0	50.0
Yield, screened pulp	%	50.5	49.0	50.9	49.9
Viscosity, CED	cP	2700	1000	2400	1300
Lignin, Halse	%	1.8	2.6	1.9	2.2
Extractives (ether and alc.)	%	0.9	0.9	0.9	0.9
Ash	%	0.5	0.5	0.5	0.4
Acetyl and formyl groups	%	0.7	1.0	0.8	0.8
Uronic acids	%	1.0	0.9	1.1	1.0
Carbohydrates, total	%	95.1	94.1	94.8	94.7
Glucan	%	80.8	85.7	82.7	86.3
		(95.7)	(98.7)	(98.5)	(100.0)
Mannan	%	9.0	4.7	7.5	5.3
		(42.2)	(21.5)	(35.5)	(24.5)
Xylan	%	5.3	3.7	4.6	3.1
		(48.7)	(33.1)	(42.7)	(28.2)

Transformation of Carbohydrates

Hägglund and co-workers have shown that the carbohydrates dissolved in the cooking liquor are slowly decomposed during the digestion. Consequently the amounts of carbohydrates found in the liquor and in the pulp do not correspond to their total amount in the original wood.

The decomposition of fermentable sugars is clearly illustrated by the results of Hägglund, Heiwinkel and Bergek.[52] Of the 218 kg of fermentable sugars present in one ton of wood 163 kg were dissolved when a strong pulp with a Roe number of 7 was produced. By taking into account the losses during the recovery of the spent sulfite liquor, those caused by fermentation and those taking place during the distillation, the ethanol yield was calculated to be 47 liters per ton of wood. Actually

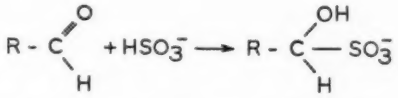
only 21.7 liters of ethanol were recovered, and hence 54 per cent of the dissolved fermentable sugars must have decomposed during the cooking process. In the cooking of a rayon pulp, the proportion of sugars decomposed was somewhat lower, 43 per cent.

Formation of Aldonic Acids

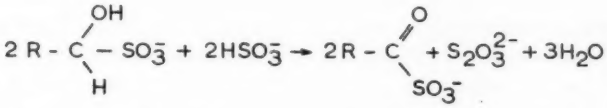
In a series of investigations Hägglund and co-workers [53] came to the conclusion that the decomposition of sugars is not dependent upon the acidity of the cooking liquor but on its calcium content, i.e. on the bisulfite ion concentration. The transformation of the sugars can thus be partly attributed to an oxidation of the aldoses to aldonic acids with the bisulfite of the cooking liquor functioning as an oxidizing agent.

$$2\,C_6H_{12}O_6 + 2\,HSO_3^- \longrightarrow 2\,C_6H_{12}O_7 + S_2O_3^{2-} + H_2O$$

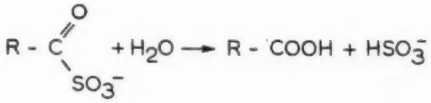
The formation of aldonic acid is believed to take place in three stages. The first stage involves the addition of the bisulfite ion to the aldose:



The α-hydroxysulfonic acid formed is oxidized by the bisulfite to an α-ketosulfonic acid and thiosulfate:



The α-ketosulfonic acid decomposes further to give aldonic acid and bisulfite:



Employing ion exchange resins, Samuelson, Ljungquist and Parck [54] have been able to isolate and determine the amounts of various aldonic acids in spent sulfite liquors. In addition to the mannonic and xylonic acids previously isolated by Hägglund and Johnson,[55] these authors isolated arabonic, gluconic and galactonic acids.

The amounts of different aldonic acids these authors found in a spent sulfite liquor are given in Table V.

Table V

Aldonic Acids Found in a Spent Sulfite Liquor According to Samuelson et al.[54]

Xylonic acid	1.72 g/l
Arabonic acid	1.35 g/l
Mannonic acid	1.44 g/l
Gluconic acid	0.31 g/l
Galactonic acid	1.60 g/l
	6.42 g/l

Samuelson [56] established that a very high proportion of the araban in wood is converted into arabonic acid. This explains why only small amounts of arabinose have been detected in spent sulfite liquors and in pulp hydrolyzates. Also a large part of the galactan is decomposed, whereas the amount of mannan decomposed represents only a small fraction of the mannan in the wood. Xylane is decomposed to a moderate extent. As only small amounts of glucose are produced in the decomposition of cellulose, the gluconic acid is produced by the degradation of wood polyoses. The gluconic acid content of the spent sulfite liquor is, however, very low. The total aldonic acids amount to about 7 per cent of the dry matter in the spent sulfite liquor. Samuelson found that about 20 per cent of the reducing sugars that are formed in the hydrolysis of wood are converted into aldonic acids. The pentoses are degraded to a greater extent than the hexoses.

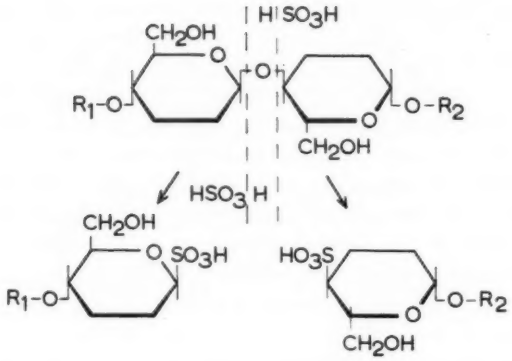
Formation of Carbohydrate Sulfonic Acids

Erdtman et al.[57, 58] (p. 71) concluded that spent sulfite liquor contains sulfonic acids derived from carbohydrates. The formation of such sulfonic acids is of practical importance for it leads to an additional consumption of sulfur during the sulfite cook and a transformation of the carbohydrates. As it is difficult to separate the carbohydrate sulfonic acids from the lignosulfonic acids, the information we have about

the former is very limited. Tsypkina and Balashova,[59] however, found that the lignosulfonic acids in spent sulfite liquor can be precipitated with hexamminecobalt chloride or nitrate, which leaves the carbohydrate sulfonic acids in solution. By this method of precipitation Kosilova and Nepenin [60] established that when spruce wood was cooked in a liquor containing 5.95 per cent total sulfur dioxide and 0.84 per cent calcium oxide, about 60 per cent of the firmly bound sulfur was present in the lignosulfonic acids and about 40 per cent in the carbohydrate sulfonic acids. The yield of the latter amounted to 6.3—12.9 per cent of the wood. By increasing the calcium oxide content of the cooking liquor to 1.35 per cent, the amount of carbohydrate sulfonic acids increased to 14.6 per cent of the weight of the wood. As they were unable to separate the carbohydrate sulfonic acids from the aldonic acids, these authors could not determine accurately the properties of the former. They did find, however, that the carbohydrate sulfonic acids were not attacked by cold or hot 2 N hydrochloric acid or by cold 2 N sodium hydroxide. The dissociation constants of these sulfonic acids were of the order of 0.1 and, in addition, the material contained also other weak acid groups, probably carboxyl groups, with dissociation constants of the order of 0.01—0.001. Also very weak acid groups were found which apparently were present in the aldonic acids.

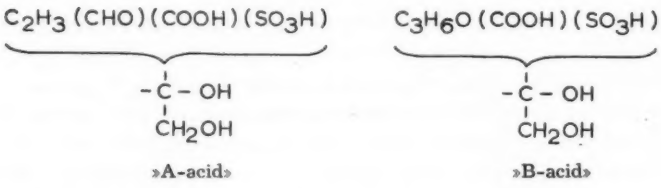
In order to avoid the interference of the lignosulfonic acids, Hägglund, Heiwinkel and Bergek [52, 61] studied the formation of carbohydrate sulfonic acids by heating holocellulose in a sulfite cooking liquor. They did not, however, obtain any accurate information about the composition or properties of these acids. When Adler [62] digested holocellulose in a cooking liquor containing 1 per cent calcium oxide and 5 per cent sulfur dioxide, he found that 45 per cent of the sugars decomposed were converted into alkali-stable carbohydrate sulfonic acids. On hydrolysis in acidic medium only an insignificant increase occurred in the copper number, which suggests that the products were not polysaccharide sulfonic acids of the type Erdtman claimed to exist in spent sulfite liquors.

Adler and Johnsson [62] attempted to prepare pure glucose sulfonic acid by heating solutions containing 2 per cent glucose, 4 per cent sulfur dioxide and various amounts of calcium oxide at 130°C, but they were not able to detect any formation of sulfonic acid. They therefore concluded that the sugars, mannose, xylose and others, formed by hydrolysis must react with bisulfite in a manner different from glucose or that the sulfonation takes place simultaneously with the degradation of the polysaccharide chain as a sulfitolysis:



According to this reaction scheme the sulfitolysis of a glucosidic bond leads to the formation of a 1-sulfonic and a 4-sulfonic acid. These sulfonic acids are then liberated from the polyose chains by hydrolysis.

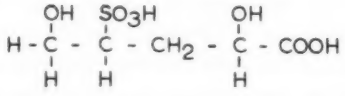
As Hägglund, Johnson and Urban [63] had shown earlier, glucose yields an alkali-stable sulfonic acid when heated in neutral sodium sulfite solutions. Adler investigated this sulfonic acid further and found it to consist of two sulfocarboxylic acids which he named »A-acid» and »B-acid» and whose structures he succeeded in clarifying to some extent:



»A-acid» »B-acid»

Of these acids the A-acid, which contains an aldehyde group, is not fully stable to alkali.

After heating xylose in a neutral sulfite solution to 135° C, Yllner [64] isolated a sulfonic acid which he identified as α, δ-dihydroxy-γ-sulfovaleric acid **(14)**.

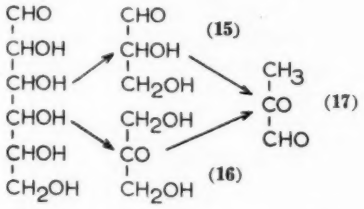


α, δ-dihydroxy-γ-sulfovaleric acid **(14)**

Formation of Methylglyoxal

Adler [65] demonstrated the occurrence of methylglyoxal in spent sulfite liquors. The amounts of methylglyoxal in three strong pulp liquors varied from 3.6 to 6 mmoles/l and the amounts in three rayon pulp liquors from 2.6 to 8.3 mmoles/l.

Earlier studies [66] had shown that hexoses are decomposed in weakly alkaline media to the trioses glyceraldehyde **(15)** and dihydroxyacetone **(16)** which are converted to methylglyoxal **(17)** by acids.



Distillation of acid or neutral monosaccharide solutions was found by Enders [67] to yield only small amounts of trioses which were transformed partly into methylglyoxal. When Adler distilled a strong pulp liquor which had been strongly acidified with sulfuric acid, he found that the yield of methylglyoxal was doubled, but similar treatment of a rayon pulp liquor did not lead to a higher yield of methylglyoxal. From this he drew the conclusion that the strong pulp liquor contained trioses in addition to methylglyoxal. In the cooking of rayon pulp, the acidity is sufficient to convert the trioses quantitatively into methylglyoxal. Adler was not able to state to what extent the trioses are present already in the wood or are formed during the cooking process. He suggested that these three-carbon compounds possibly represent an intermediate stage in the formation of carboxylic and sulfonic acids. It is further possible that an oxidative degradation of methylglyoxal by bisulfite gives rise to a part of the acetic acid found in spent sulfite liquor.

Formation of Carbon Dioxide

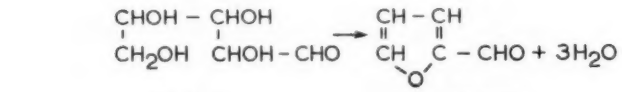
Large quantities of carbon dioxide are liberated during the sulfite cooking process. In laboratory experiments Routala et al.[68, 69, 70] found that the carbon dioxide is produced when the hemicellulose components decompose.

When spruce wood was treated with a sulfite cooking liquor low in calcium (1.04 % CaO), the amount of carbon dioxide liberated cor-

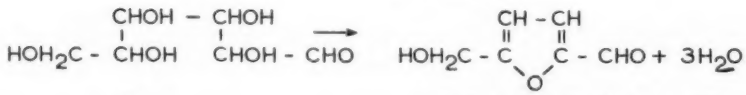
responded to 0.58 per cent of the wood, but when the liquor contained more calcium (1.51 % CaO), the amount of carbon dioxide liberated increased to 0.84 per cent of the wood. Routala showed further that heating of calcium gluconate with a sulfite solution gives carbon dioxide and a pentose, probably arabinose, which is then converted into furfural. Accordingly, the formation of carbon dioxide may be connected with aldonic acid formation and sugar decomposition.

Formation of Furfural and 5-Hydroxymethylfurfural

Pentoses, when heated in an acid medium, are converted into furfural **(18)**, whereas hexoses yield 5-hydroxymethylfurfural **(19)**:



Pentose Furfural **(18)**



Hexose 5-Hydroxymethylfurfural **(19)**

When he studied the formation of furfural during the sulfite cook, Gadd [71] found that the furfural (and 5-hydroxymethylfurfural) content increased toward the end of the cook.

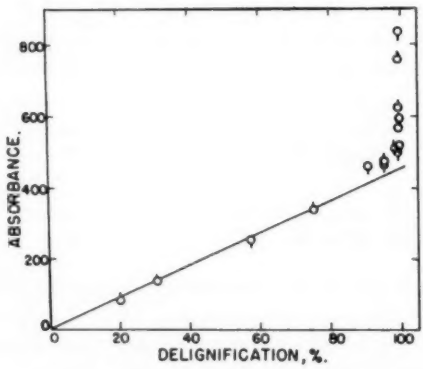


Fig. 3. Relation between percentage delignification of hemlock and solution absorbance at 2800 Å. Cooking liquor contained: ♂, 4.0 % »free» SO_2; ♀, 8.0 % »free» SO_2. According to McCarthy et al.[72]

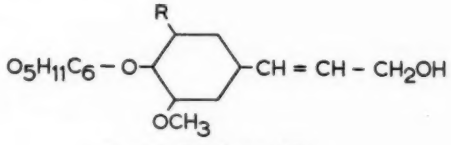
Furfural gives an ultraviolet absorption spectrum that resembles that of lignin. When the absorbance at 280 mμ is plotted against the delignification expressed as a percentage of eliminated methoxyl groups, a straight line is obtained up to a delignification of 90—95 per cent, but the absorbance then increases strongly (Fig. 3). McCarthy et al.[72] concluded that the increase in the absorbance may be due to hydrolysis or condensation reactions of lignin or to the formation of furfural and 5-hydroxymethylfurfural.

Gadd's result suggests that the increased absorption is due to the formation of furfural and 5-hydroxymethylfurfural. The amounts of furfural he found in various technical spent sulfite liquors varied from 0.02 to 0.06 per cent.

LIGNIN

THE BIOSYNTHESIS OF LIGNIN

In 1875 Tiemann [74] determined the constitution of the glucoside coniferin (**20**) which appears in the cambial sap of conifers during the growth period.



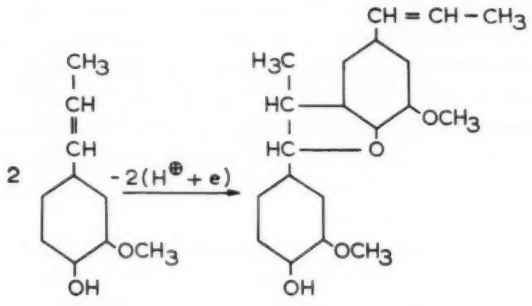
R = H Coniferin (**20**)
R = OCH_3 Syringin (**21**)

In the cambial sap of deciduous trees, coniferin is accompanied by the glucoside syringin (**21**).

Tiemann and Mendelsohn [75] assumed that coniferin and lignin are related compounds and in 1897 Klason [76] published a theory according to which lignin is related structurally to coniferyl alcohol.

Cousin and Hérissey [77] oxidized isoeugenol (**22**) with mushroom oxidase to a compound which Erdtman [78] found to be a phenylcoumaran (**23**) similar to that which Freudenberg assumed to exist in lignin.

Erdtman proposed that lignin is formed by the oxidative polymerization of guaiacylpropane derivatives. This view was accepted by Freudenberg who studied the biosynthesis of lignin in an extensive series of investigations.



(22) (23)

Freudenberg introduced various radioactive compounds into young spruce trees by allowing solutions of the radioactive compounds to enter the trees through cut needles. When the trees took up radioactive D-coniferin, Freudenberg found the lignin to be radioactive. The introduction of radioactive L-coniferin did not impart radioactivity to the lignin.[79]

Radioactive phenylalanine was converted into radioactive coniferin during the course of one or two days. A few days later radioactive lignin could be isolated from a region which was lignified at the time of the experiment.[80]

During the growth period the cambium and the adjoining cells contain large amounts of coniferin and laccase and small amounts of peroxidase.[81]

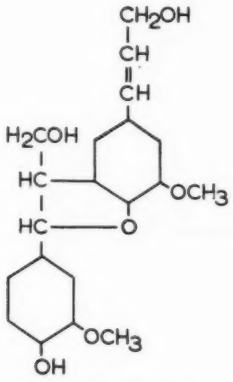
Freudenberg was not, however, able to find any β-glucosidase which is necessary for the degradation of coniferin in the cambial zone, but did find it in a zone between the young and lignified cells. He hence concluded that coniferin diffuses into newly formed cells where it is hydrolyzed to coniferyl alcohol, and this is then converted into lignin by laccase and peroxidase.

The lignification of secondary wood takes place mainly in the compound middle lamella. In primary wood, however, only the secondary walls are lignified. It is hence possible that, in contrast to Freudenberg's opinion, the lignin precursors are not produced in the meristematic regions but within the cells.[82]

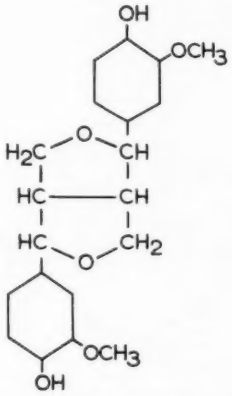
Freudenberg also attempted to convert coniferyl alcohol into lignin in vitro.[83] By aerating solutions of coniferyl alcohol containing the mushroom dehydrase laccase,[81] he obtained an insoluble precipitate which he took to be identical with natural lignin. Instead of mushroom

laccase, cambium laccase can be employed in acid solution or peroxidase in the presence of a low concentration of hydrogen peroxide. The reaction takes place also in the presence of copper sulfate and oxygen.

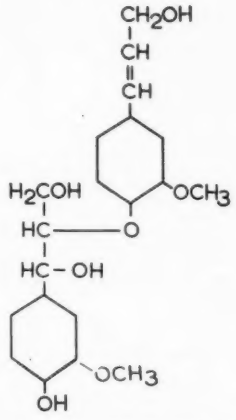
If the reaction is terminated too early, more than twenty low-molecular compounds can be isolated which must be considered intermediate products in the formation of lignin. Three of the most important of these compounds are dehydrodiconiferyl alcohol **(24)**, DL-pinoresinol **(25)** and guaiacylglycerol-β-coniferyl ether **(26)**.



Dehydrodiconiferyl alcohol **(24)**

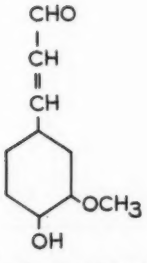


DL-Pinoresinol **(25)**

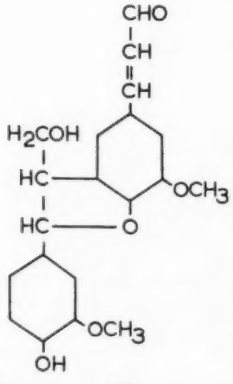


Guaiacylglycerol-β-coniferyl ether **(26)**

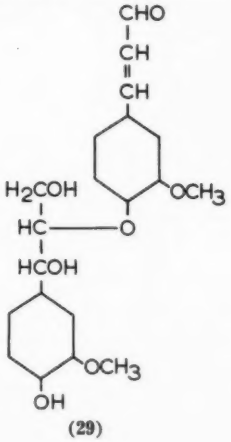
Also the following compounds have been isolated in small quantities.



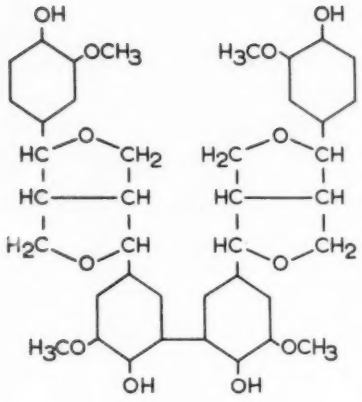
Coniferyl aldehyde **(27)**



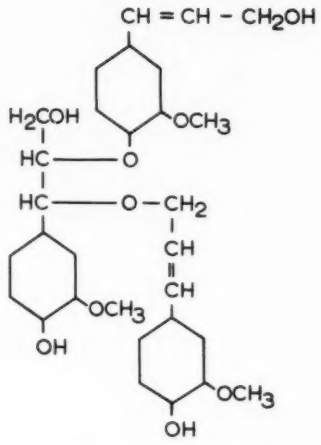
(28)



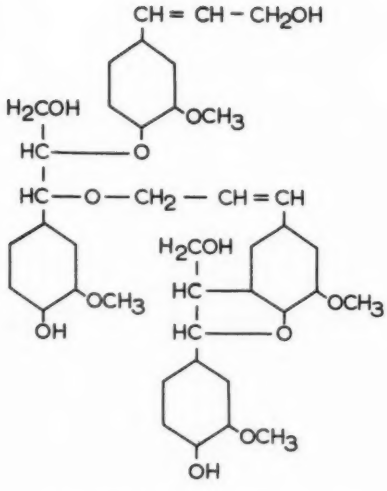
(29)



Dehydrodipinoresinol (30)

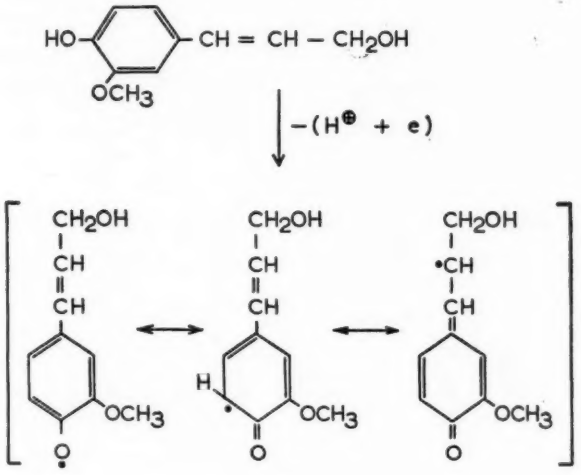


Guaiacylglycerol-α,β-bis-coniferyl ether (31)



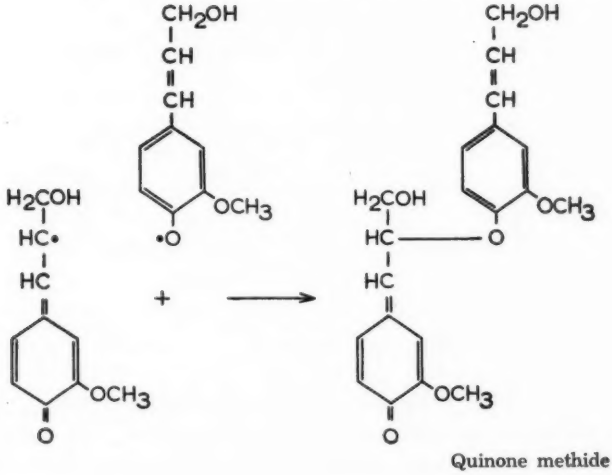
Guaiacylglycerol-α-dehydrodiconiferyl-β-coniferyl ether (32)

Freudenberg's view is that lignin formation begins with an enzymatic dehydrogenation of coniferyl alcohol which leads to the formation of a mesomeric radical **(33)**:



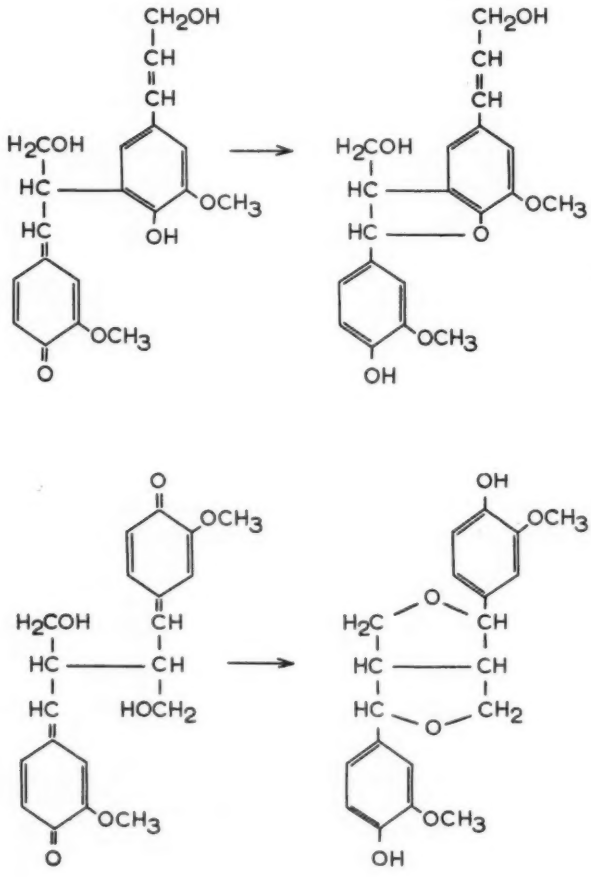
(33)

By the coupling of two radicals with extreme limiting mesomeric structures there result dimeric intermediate products that contain quinone methide groups such as, for example, the following **(34)**:

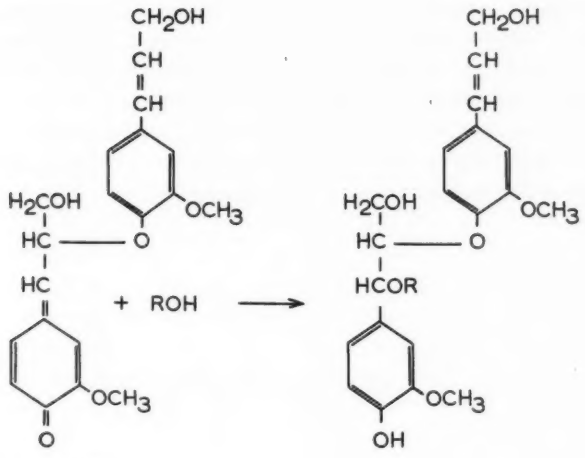


Quinone methide **(34)**

The quinone methides that are produced by β,ortho- and β,β-coupling are stabilized by intramolecular rearrangement with the formation of dehydrodiconiferyl alcohol and DL-pinoresinol:



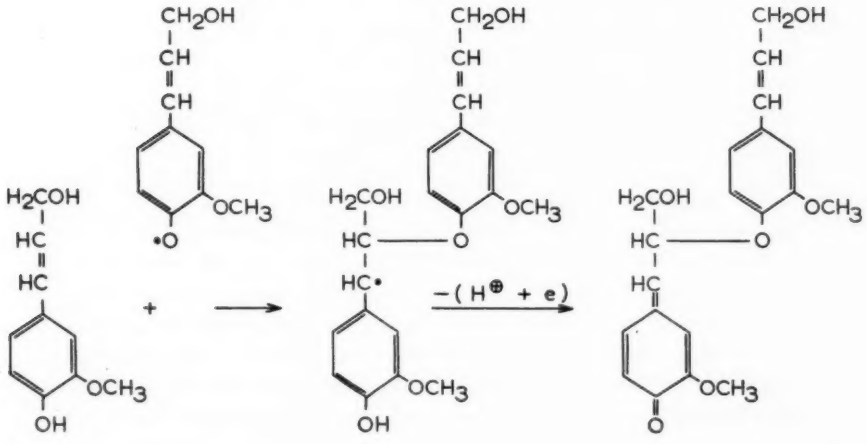
The dimer that is formed by β,aroxyl-coupling cannot, however, become stabilized by intramolecular rearrangement, but takes up one molecule of water to form guaiacylglycerol-β-coniferyl ether **(26)**.



R = H (26)
R = CH_3 (35)
R = $C_{12}H_{21}O_{10}$ (36)

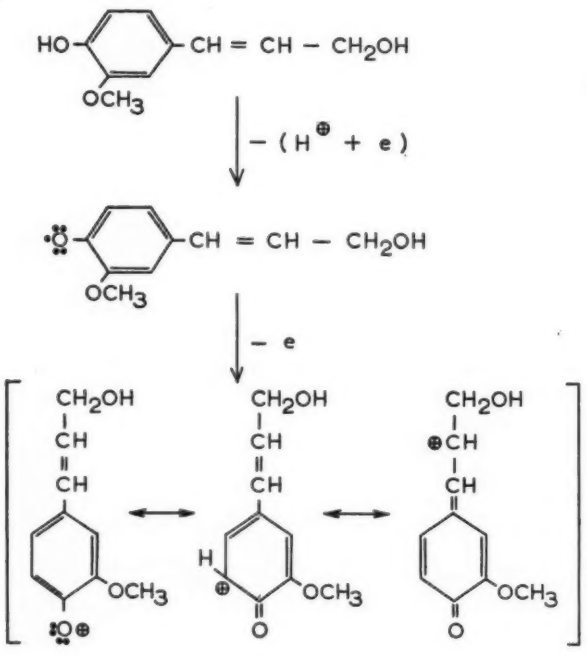
These dimeric phenols may then react further in the same way as coniferyl alcohol.

Instead of a coupling of radicals, there may according to Adler [84] occur a reaction between one radical and a coniferyl alcohol molecule. After the elimination of a hydrogen atom, the same dimer will result as from the coupling of two radicals.



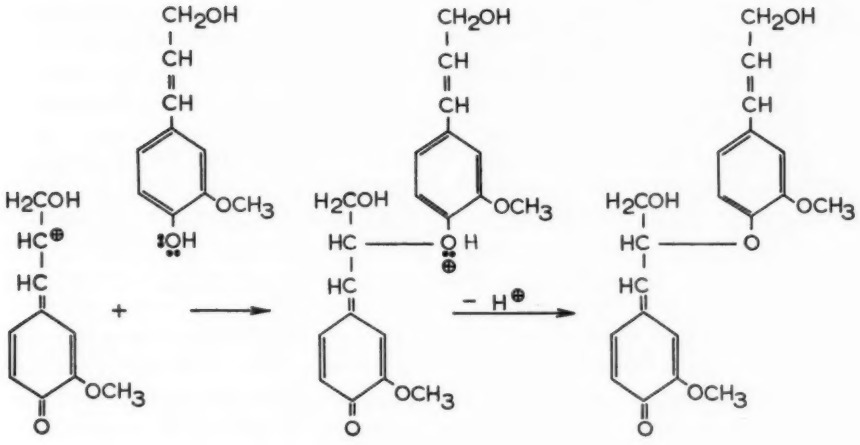
The dimeric radical may, however, react further with coniferyl alcohol, but Adler's opinion is that this non-oxidative polymerization mechanism does not predominate, for the reactivities of the radicals formed should be reduced by resonance stabilization.

Adler [84] has also proposed for the initial reaction another course in which the unpaired electrons of the radicals are removed by the enzyme whereupon a mesomeric cation **(37)** is produced.



(37)

This cation then reacts with a coniferyl alcohol molecule to form the same dimer as is formed by the coupling of two radicals:



If lignin is formed by a dehydrogenation mechanism only, two hydrogen atoms should be released for each phenylpropane unit in an infinite chain. In addition, 0.4 hydrogen atom should be eliminated for each 0.2 carbonyl group per phenylpropane unit which is formed in the lignification according to Adler. The total hydrogen loss is, however, almost two atoms per phenylpropane unit. The coniferyl alcohol thus loses only 1.6 hydrogen atoms in the formation of the uniting bonds. As Freudenberg et al. have pointed out, this means that one of five linked units is bound without the elimination of hydrogen.[85]

A non-oxidative polymerization mechanism suggested by Adler was mentioned already on p. 40. Freudenberg, however, believes that the addition of alcohols to the quinone methides formed represents the non-oxidative growth mechanism. Freudenberg and Grion [86] found that guaiacylglycerol-α-methyl-β-coniferyl ether **(35)** is formed when coniferyl alcohol is dehydrogenated enzymatically in an aqueous solution containing 30 per cent methanol. They also prepared the ether by oxidizing coniferyl alcohol with manganese dioxide in methanol. When coniferyl alcohol was dehydrogenated enzymatically in an aqueous solution containing 66 per cent cane sugar, a compound **36** was detected by paper chromatography. This compound which could be prepared in a nonaqueous medium contained one molecule of sucrose to 2—4 coniferyl alcohol molecules. Freudenberg concluded this compound to be an etherlike sugar compound and to represent a model for the lignin-carbohydrate complex.

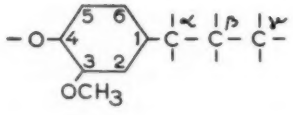
At an early stage of the dehydrogenation of coniferyl alcohol a compound is formed which is hydrolyzed by water to guaiacylglycerol-β-coniferyl ether and coniferyl alcohol. The structure **31** has been proposed for this compound.[87] Also a compound assumed to have the structure **32** that hydrolyzes to **24** and **26** was isolated.

Adler,[84] however, found it difficult to understand how the intramolecular rearrangement of carbinol hydroxyl groups could take place in vivo in the presence of a large excess of water. The formation of benzyl alcohol would be expected to overweigh the non-enzymatic formation of a benzyl alkyl ether. An enzymatic coupling of carbinol groups is also improbable, for it should lead to optically active benzyl ethers, whereas lignin is optically inactive. He concluded that it is unwarranted to assume the existence of benzyl alkyl ether bridges in lignin if a more specific formation mechanism cannot be postulated. He stressed that it has not yet been possible to demonstrate that the frequently postulated benzyl alkyl ether bonds actually occur in lignin. However, the benzyl alcohol group has been shown to exist in lignin by means of the quinone-chlorimide reaction (p. 45).

THE COMPOSITION OF LIGNIN

Björkman lignin (MWL-milled wood lignin) [88, 89] is the lignin preparation which most closely resembles the protolignin of wood.

On the assumption that lignin is composed of phenylpropane units,



an elementary analysis [90] gave for the composition of a Björkman lignin when the 1.9 per cent of carbohydrates present were subtracted the following:

$$C_9H_{8.83}O_{2.37}(OCH_3)_{0.96}$$

By taking into account various analytical data, Adler [91] allocated the oxygen atoms to various functional groups and came to the following distribution:

$$C_9H_{7.68}\ (\text{Phenolic OH})_{0.29}(\text{Aliphatic OH})_{0.86}(\text{Carbonyl O})_{0.18}$$
$$(\text{Aryl alkyl ether O})_{0.71}(\text{Dialkyl ether O})_{0.33}(OCH_3)_{0.96}$$

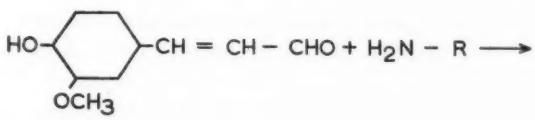
Phenolic Hydroxyl Groups

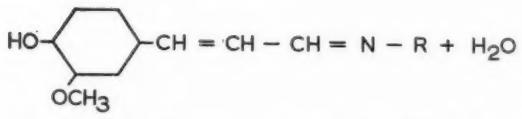
The previously greatly debated question of the number of phenolic hydroxyl groups in lignin must now be considered satisfactorily solved. By potentiometric titration of Björkman lignin in ethylenediamine solution with sodium colamine, Freudenberg and Dall [92] obtained the value 0.33 hydroxyl group per methoxyl group and by titration in dimethyl formamide solution with tetramethylammonium hydroxide, Enkvist, Alm and Holm [93] obtained the value 0.38 hydroxyl group per methoxyl group. By oxidative demethylation with sodium periodate, Adler et al.[94] obtained the value 0.30 OH/MeO. Brauns' native lignin they found to contain a higher proportion of phenolic hydroxyl groups or 0.46 OH/MeO.

Freudenberg and Dall [92] determined the phenolic hydroxyl groups in a lignosulfonic acid by potentiometric titration and obtained the value 0.43 OH/MeO. When the same preparation was analyzed by the photometric method of Aulin-Erdtman,[95] the result was only 0.23—0.34 OH/MeO and when analyzed by the similar method of Goldschmid,[96] 0.28 OH/MeO. In the photometric method the phenolic hydroxyl content is determined from the difference in the ultraviolet absorption in neutral and alkaline solution. In certain cases, however, this method may give low values. For example, Lindberg and Enkvist [97] have found that it cannot be applied to orthosubstituted phenols such as salicylic acid and o-vanillic acid. Also only about half of the phenol content obtained by titration of lignothioglycolic acid is analyzed by the method.[85]

Carbonyl Groups

Lignin contains a low proportion of coniferyl aldehyde groups that react with aromatic amines to Schiff bases which are intensely yellow in color:



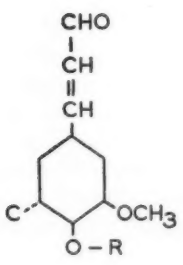


The coniferyl aldehyde group content can be estimated from the

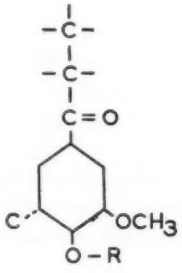
absorbance at 445 mμ after reaction with p-aminobenzoic acid.[98, 99] Brauns' native lignin and Björkman lignin were found by this method to contain one coniferyl aldehyde group to about 35 phenylpropane units.[100] Lignosulfonic acid, on the other hand, contains only one coniferyl aldehyde group to 40—60 phenylpropane units [101] because the aldehyde groups are partly destroyed during sulfite digestion (p. 81).

By comparing the changes in the ultraviolet spectrum of Björkman lignin produced by reducing the latter with sodium borohydride, Adler and Marton [102] concluded that the lignin contained the following numbers of carbonyl groups per methoxyl group:

< 0.01 phenolic coniferyl aldehyde group **(38)**
0.03 etherified coniferyl aldehyde group **(39)**
0.005—0.01 phenolic 4-ketoguaiacol group **(40)**
0.05—0.06 etherified 4-ketoguaiacol group **(41)**

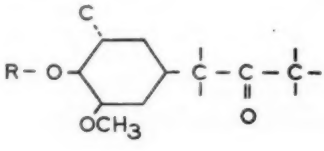


R = H **(38)**
R = the side chain of a second phenylpropane unit **(39)**



R = H **(40)**
R = the side chain of a second phenylpropane unit **(41)**

The 0.09—0.11 conjugated carbonyl group per methoxyl group represents about half the total carbonyl content (0.18—0.21 CO/MeO) as determined by the hydroxylamine hydrochloride method.[103] Adler and Marton ascribed the difference to the existence of β-keto groups **(42)**.

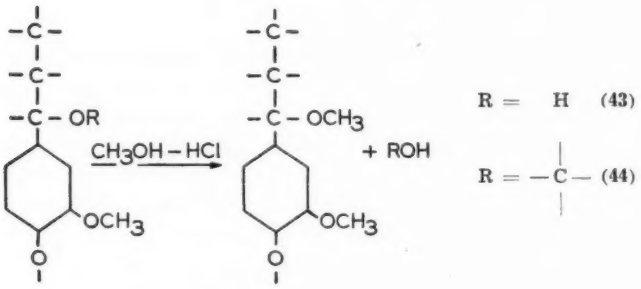


(42)

Aliphatic Hydroxyl Groups and Ether Linkages

Substituents on the α-Carbon Atom

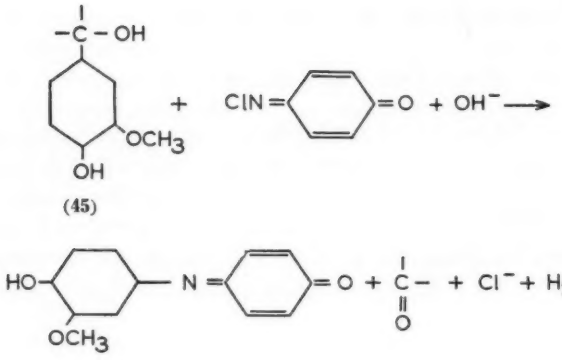
Adler and Gierer [104] allowed Brauns' native lignin to react with methanol containing 0.5 per cent hydrogen chloride and found that approximately every other guaiacylpropane unit combined with a methyl group. They were able to show that the reaction probably involved the etherification of a guaiacylcarbinol group **(43)** or a transetherification of a guaiacylcarbinol ether **(44)**.



Adler [84] treated Björkman lignin with methanol-dioxane (1:1) containing 0.5 per cent hydrogen chloride. He found that 0.42 methoxyl group per phenylpropane unit had been taken up in the etherification of benzyl alcohol groups and small numbers of coniferyl alcohol groups and by a transetherification of the corresponding ether groups. By model experiments he found that the benzyl ether structure in pinoresinol dimethyl ether in which the phenol hydroxyl groups were etherified and the cyclic benzyl aryl ether bonds in dehydrodiconiferyl alcohol did not react under the same conditions.

Gierer [105, 106] has definitely established by the quinone monochlorimide reaction that phenolic benzyl alcohol groups **(45)** are present in lignin.

According to Adler [84] only one phenolic guaiacylcarbinol group is found for approximately one hundred phenylpropane units in Björkman lignin. Either the phenolic hydroxyl groups or the benzyl alcohol groups, or both these groups, in the greater part of the phenylpropane units must thus be etherified. Brauns' native lignin, on the other hand, contains one phenolic guaiacyl carbinol group to 7—8 phenylpropane units.[105]



(45)

By the aid of model experiments, Adler [84] showed that the acyclic benzyl ethers in lignin must be dialkyl ethers because the content of phenolic hydroxyl groups did not appreciably increase in number when lignin was transetherified with methanolic hydrochloric acid.

Substituents on the β-Carbon Atom

In a series of investigations Hibbert et al.[107, 108, 109] obtained the following four ketones in a total yield of about 5 per cent when they heated spruce wood flour with hydrochloric acid:

$R—CO—CH(OC_2H_5)—CH_3$ (46)

$R—CO—CO—CH_3$ (47)

$R—CH(OC_2H_5)—CO—CH_3$ (48)

$R—CH_2—CO—CH_3$ (49)

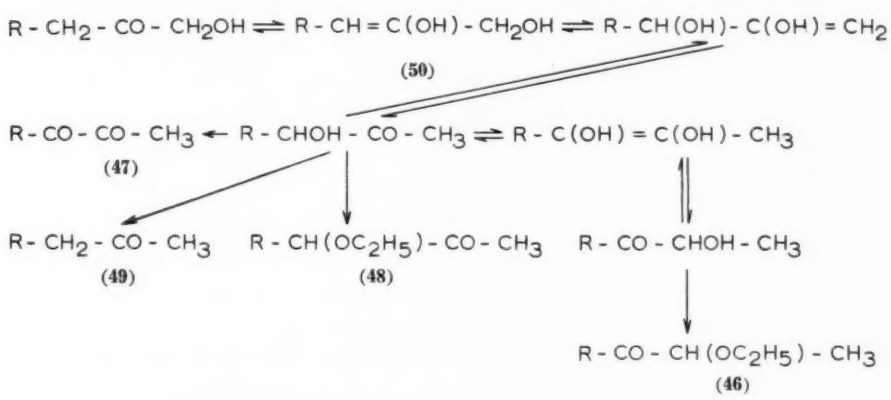
R = 4-hydroxy-3-methoxyphenyl

From maple wood they obtained the corresponding syringyl derivatives (R = 4-hydroxy-3,5-dimethoxyphenyl).

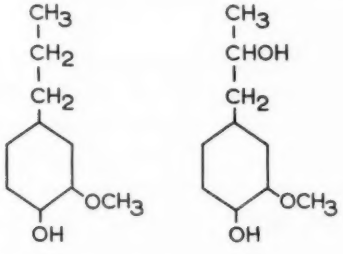
These ketones lack the primary alcohol groups or terminal ether groups which have been found by analysis to exist in lignin.[110, 111] It may also be mentioned that 3-cyclohexyl-1-propanols have been produced by the high pressure hydrogenation of lignin.[112, 113, 114]

Hibbert therefore assumed that the ketones (46—49) are formed

by a series of rearrangements from a structural unit of the β-hydroxy-coniferyl alcohol type **(50)**:

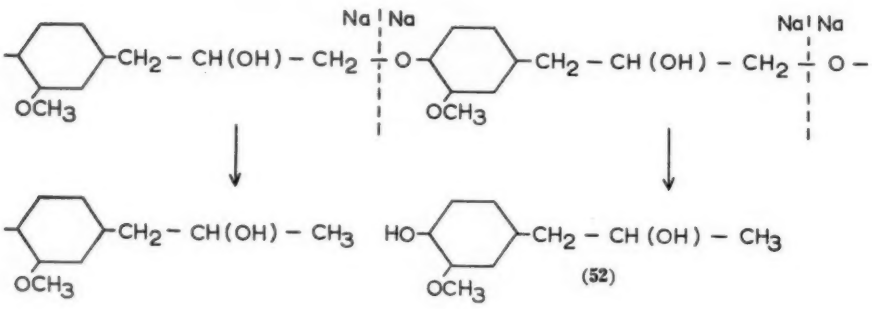


This view was accepted by Schorygina, Semechkina and Kefeli [115] who cleaved the ether bonds of cuoxam lignin and other lignin preparations with sodium metal in liquid ammonia at —33°C. In this way they were able to cleave lignin almost quantitatively into low-molecular compounds of which 28 per cent were aromatic monomers. Of these dihydroeugenol **(51)** and 1-(4-hydroxy-3-methoxyphenyl)-propanol-2 **(52)** amounted to 6.7 and 12 per cent of the original lignin.



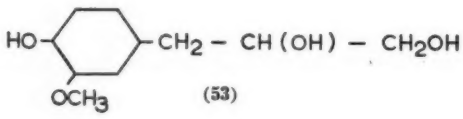
Schorygina, Semechkina and Kefeli ascribed the high breakdown to the fact that a large part of the structural elements of lignin are bound to each other primarily by ether bonds, but they do not deny the possibility that lignin may also contain some carbon-carbon bonds between phenylpropane units. The apparent stability of lignin to hydriodic acid Nikitin [116] explained by stating that this acid not only ruptures ether bonds but also brings about a condensation of the lignin.

Schorygina, Semechkina and Kefeli proposed the following reaction scheme for the formation of 1-(4-hydroxy-3-methoxy-phenyl)-propanol-2 **(52)** by the action of sodium on lignin in liquid ammonia:



(52)

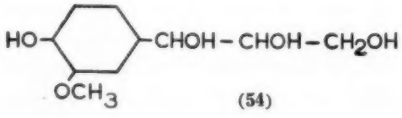
The basic unit of lignin can according to Schorygina et al. be β-hydroxyhydroconiferyl alcohol **(53)** or more probably, in agreement with Hibbert, β-hydroxyconiferyl alcohol **(50)**.



(53)

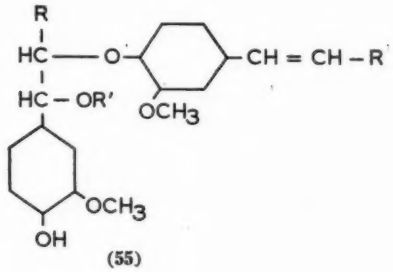
Adler and Gierer,[117] however, presented two reasons why β-hydroxyconiferyl alcohol groups cannot be components of lignin. The enol form should lead to a greater number of double bonds conjugated with the aromatic nucleus than has been found spectrophotometrically. The keto form, on the other hand, should be ruptured by periodate. Actually, however, also periodate lignin can yield Hibbert's ketones.

It was likewise possible to conclude that no glycerol side chain **(54)** existed. Although such compounds decompose to Hibbert's ketones, they, in contrast to lignin, yield large amounts of formaldehyde when they are oxidized with periodate.[118]



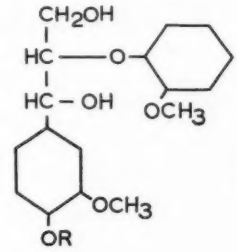
(54)

Ether formation between the β-carbon atom and the phenolic hydroxyl group **(55)** was suggested by Erdtman and Leopold.[119]



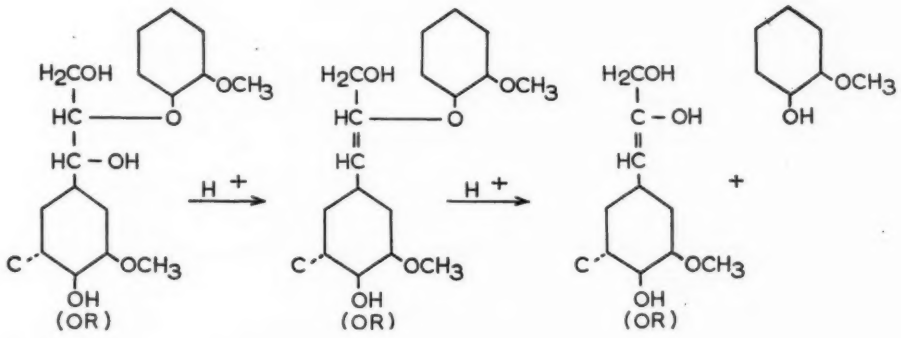
(55)

Adler et al.[120, 121] also succeeded in converting guaiacylglycerol-β-guaiacyl ether. **(56)** and veratrylglycerol-β-guaiacyl ether **(57)** into Hibbert's ketones.



R = H **(56)**
R = CH_3 **(57)**

These authors therefore considered it probable that guaiacylglycerol-β-aryl ethers are the units in lignin that yield Hibbert's ketones. They proposed the following course for the reaction:



4

β-Hydroxyconiferyl alcohol then reacts as described by Hibbert (p. 47).

As already mentioned on p. 35, guaiacylglycerol-β-coniferyl ether is one of the most important dimers formed in the enzymatic dehydrogenation of coniferyl alcohol.

Substituents on the γ-Carbon Atom

When heated in the presence of strong mineral acids, lignin yields 1.5—2 per cent formaldehyde. Experiments with model substances and Freudenberg's »Dehydrierungspolymerisat» (DHP) showed that the formaldehyde may be derived from the primary alcohol group of coniferyl alcohol.[122]

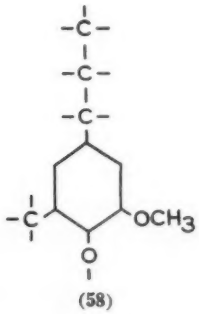
DHP prepared from coniferyl alcohol with a radioactive carbon atom in the γ-position yields radioactive formaldehyde which thus has its origin in the primary hydroxyl group.[123]

Ether bonds at the γ-carbon atom are primarily found in the pinoresinol units and in the units **31** and **32** (p. 35).

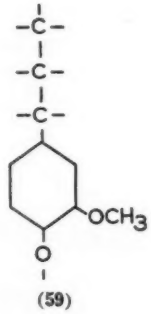
Carbon-Carbon Bonds between Phenylpropane Units

Substitution on the Aromatic Nucleus

In addition to the ether bonds, various carbon-carbon bonds have been postulated between the phenylpropane units. Of these, the bonds uniting the phenylpropane units through carbon atom 5 in the aromatic nucleus have aroused the greatest interest. The resulting structures have been called condensed **(58)** whereas phenylpropane structures lacking these bonds are referred to as being open **(59)**:



(58)



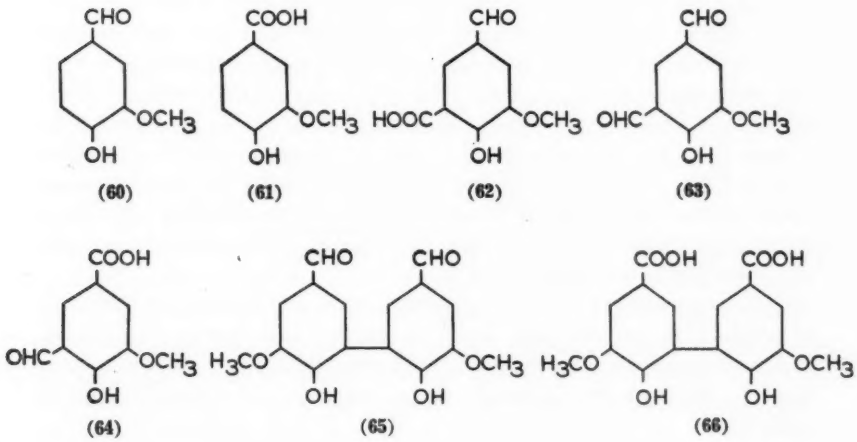
(59)

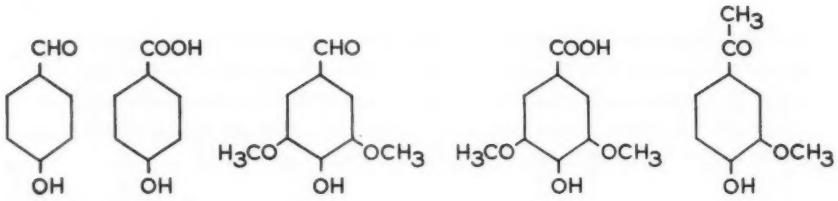
The existence of open and condensed structures in lignin has been studied primarily by oxidation of lignin with nitrobenzene and alkali.[124, 125, 126] Leopold [127] oxidized spruce wood by this procedure and obtained the products mentioned in Table VI. The yields are given as percentages by weight of the lignin content of the wood.

Table VI

Oxidation Products of Spruce Wood (*Picea excelsa*) According to Leopold [127]

Vanillin **(60)**	27.5	%
Vanillic acid **(61)**	4.8	%
5-Carboxyvanillin **(62)**	1.2	%
5-Formylvanillin **(63)**	0.23	%
5-Formylvanillic acid **(64)**	~ 0.1	%
Dehydrodivanillin **(65)**	0.80	%
Dehydrodivanillic acid **(66)**	0.03	%
p-Hydroxybenzaldehyde **(67)**	0.25	%
p-Hydroxybenzoic acid **(68)**	+	
Syringaldehyde **(69)**	0.06	%
Syringic acid **(70)**	~ 0.02	%
Acetoguaiacone **(71)**	0.05	%





(67) (68) (69) (70) (71)

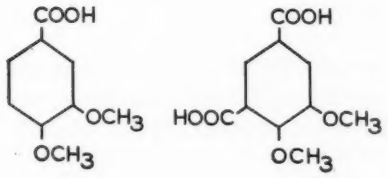
The table shows that the proportion of reaction products that contain only one carbon-aryl bond is about 33 per cent. If the yield of the reaction is from 75 to 90 per cent, this means that 45—55 per cent of the phenylpropane units have no substituents at carbon atom 5.[128]

If the formation of vanillin requires the existence of free phenolic hydroxyl groups, the theoretical yield based on Enkvist and Adler-Hernestam's data (p. 43) would be 30—35 per cent for Björkman lignin. This presumes further that the free phenolic hydroxyl groups are bound to open units. According to Lautsch, [129] however, the ether linkages are cleaved before the oxidation and this has been confirmed by Leopold [130] by means of model experiments.

Treatment with alkali, however, has been shown to have a negative effect, for Pew [131] found that spruce wood which yielded 26 weight per cent vanillin when subjected to alkaline nitrobenzene oxidation gave only 14 per cent vanillin when it was heated under pressure in 2 N sodium hydroxide prior to the oxidation.

Leopold's opinion that 45—55 per cent of the phenylpropane units in lignin are open is valid only on the assumption that the bonds at carbon atom 5 are not cleaved so that the vanillin yield is thereby increased. That this does not take place has been demonstrated by numerous model experiments. The yield of vanillin was quite low, but the yield of 5-formylvanillin and 5-carboxyvanillin varied from 19 to 38 per cent.[130] The substituent at carbon atom 5 can, however, be removed in certain cases. Thus Pew [131] was able to obtain vanillin in 9.7 per cent yield from eugetic acid. However, the yield of 5-carboxyvanillin at the same time was as high as 65 per cent. Dehydrodiisoeugenol gave approximately the same amount of vanillin as spruce lignin but much more 5-carboxyvanillin. From this Pew concluded that most of the phenylpropane units in spruce lignin are of the open type.

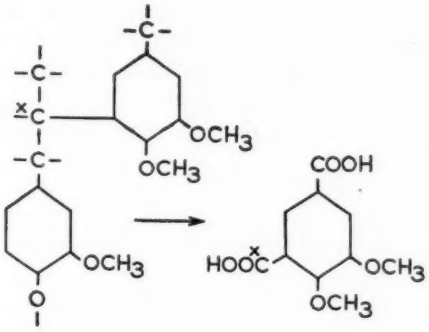
When wood is methylated with diazomethane and then oxidized with permanganate, veratric **(72)** and isohemipinic **(73)** acids are obtained in 4.9 per cent and 0.9 per cent yields, respectively, on the lignin content.[132] This reaction suggests the existence of condensed phenolic units also.



(72) (73)

By preliminary treatment of wood with alkali the yield of isohemipinic acid can be increased to 6—10 per cent. Richtzenhain attributed this to the condensation of the decomposition products of carbohydrates with phenylpropane units at position 5 in the latter.

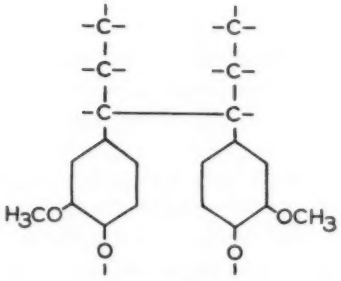
Attempts have also been made to determine how the carbon atom 5 is combined in the condensed units. By enzymatic oxidation of coniferyl alcohol labelled with radioactive carbon (^{14}C) in the β-position, Freudenberg and Niedercorn [133] obtained a synthetic lignin which, when oxidized by permanganate after methylation, yielded radioactive isohemipinic acid:



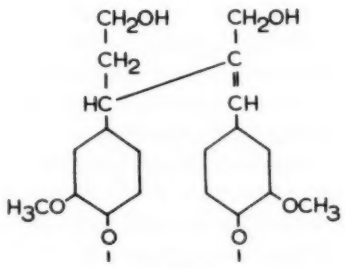
As shown by the data in Table VI, also dehydrodivanillin and dehydrodivanillic acid are formed when wood is oxidized by nitrobenzene. Pew [131] obtained much more (2.2 %) dehydrodivanillin from spruce wood. Brauns' native lignin also gave 2.2 per cent dehydrodivanillin. Aulin-Erdtman [134] estimated by a spectrophotometric method that the diphenyl units which contain free phenolic hydroxyl groups amount to 5 per cent in Brauns' lignin from spruce, whereas the content is lower in other lignins. As dehydrodivanillin was produced only from model substances that contained diphenyl groups, Pew concluded that lignin contains such groups.

Bonds between Side Chains

By oxidation of spent sulfite liquor with alkaline copper(II)oxide, Pearl and Beyer [135] obtained small amounts of reaction products that pointed to the existence of α,α-bonds in lignosulfonic acids.



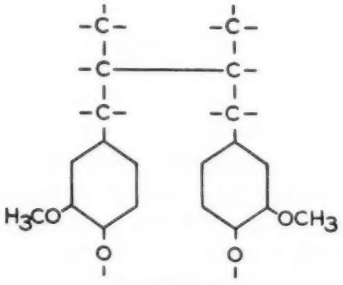
α,α-bond



α,β-bond

Freudenberg et al.[136, 137] have suggested that carbon-carbon bonds also occur between the α- and β-carbon atoms of the side chains of two phenylpropane units. He assumed the bond to be formed by spontaneous dimerization of coniferyl alcohol groups.

The β,β-bond of lignans is characteristic of the pinoresinol structure of lignin.



β,β-bond

By treating lignin with sodium in ammonia at a low temperature, Schorygina et al.[138] obtained a compound which they believed to be 2,3-bis-(hexahydrobenzyl)-butane in a yield of about 0.4 per cent.

Methoxyl Groups

The methoxyl groups in lignin can be thought to exist as aliphatic or phenolic ether groups, as acetal methoxyl groups or as ester groups. As, however, the methoxyl groups in lignin are not readily eliminated by heating the lignin in dilute acid or alkali, they can hardly occur as acetal or ester groups.[139] The methyl groups can, however, be eliminated by heating with hydriodic acid. By comparing the rate of elimination of methoxyl groups from lignin and other compounds, Freudenberg et al.[140] demonstrated that the methoxyl groups in lignin are bound to aromatic nuclei.

If lignin is assumed to comprise only guaiacylpropane units, it should contain one methoxyl group to each phenylpropane unit. Actually, however, it has never been possible to demonstrate more than about 0.96 methoxyl group per phenylpropane unit in Björkman lignin.[90] Leopold [127] (p. 51), who isolated small amounts of p-hydroxyphenyl units from spruce wood, supposed that the deficiency of methoxyl groups may be due to the occurrence of methoxyl-free phenylpropane units in the lignin.

In this connection it may be mentioned that the determination of the methoxyl contents of lignosulfonic acids may be complicated by the reduction of the sulfur in the sulfonic acid groups to hydrogen sulfide which reacts with the methyl iodide formed:[141, 142, 143]

$$CH_3J + H_2S \rightarrow CH_3SH + HJ$$

$$CH_3SH + CH_3J \rightarrow CH_3SCH_3 + HJ$$

THE PROTOLIGNIN OF WOOD AND FREUDENBERG'S DIMERS

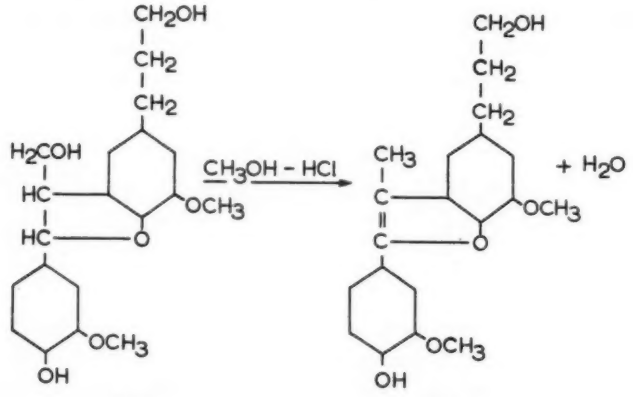
As mentioned previously (p. 35) the three main intermediate compounds formed when coniferyl alcohol is dehydrogenated enzymatically are dehydrodiconiferyl alcohol **(24)**, DL-pinoresinol **(25)** and guaiacylglycerol-β-coniferyl ether **(26)**. It is therefore of some interest to know how large amounts of these units may be present in lignin.

Pinoresinol Units

There is very little data on the amounts of pinoresinol units that occur in lignin. When pinoresinol is methylated with methanolic hydrogen chloride, it takes up about 70 per cent of the calculated maximum amount of methoxyl, whereas pinoresinol dimethyl ether does not react with the reagent. The difference in the degree of methylation of Björkman lignin before and after methylation with diazomethane Adler [84] took to indicate that Björkman lignin contains at most 10 per cent phenolic pinoresinol units.

Dehydrodiconiferyl Alcohol Units

More information is available on the content of dehydrodiconiferyl alcohol units in lignin. Adler et al.[144, 145] found that dihydro-dehydrodiconiferyl alcohol **(74)** is transformed by heating with methanolic hydrogen chloride into a phenylcoumarone derivative **(75)** that strongly absorbs ultraviolet light with a maximum absorption at 310 mμ.

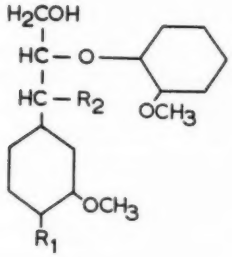


(74) **(75).**

The increase in the absorption at 310 mμ of Björkman lignin that had been heated in methanolic hydrogen chloride indicated that approximately 18 per cent of the phenylpropane units were present as phenylcoumarans. The majority of these (16 per cent) were found to have free phenolic hydroxyl groups.

Guaiacylglycerol-β-coniferyl Ether Units

The observation that the model compound veratrylglycerol-β-guaiacyl ether (**57**) is sulfonated by sulfite solutions to **76**, is methylated by methanolic hydrogen chloride at room temperature to **77**, and yields Hibbert's ketones on alcoholysis (p. 49) [146, 120, 147] strongly supports the view that lignin contains structural units of the guaiacylglycerol-β-coniferyl ether type.

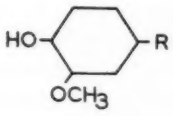


$R_1 = OCH_3$ $R_2 = OH$ **(57)**

$R_1 = OCH_3$ $R_2 = SO_3H$ **(76)**

$R_1 = OCH_3$ $R_2 = OCH_3$ **(77)**

Adler et al. showed also that if the model substance is heated under reflux with a dioxane-water (9:1) mixture that is 0.2 N in hydrogen chloride, guaiacol is liberated and the ketones **78**, **79** and **80** are formed.



$R = CO - CH(OH) - CH_3$ **(78)**

$R = CO - CO - CH_3$ **(79)**

$R = CH_2 - CO - CH_3$ **(80)**

The acidolysis of dioxane-hydrochloric acid lignin and Björkman lignin also yields the ketones **78—80** and a water-insoluble residue. As stated previously on p. 49, the reaction takes place according to Adler et al.[121] with the liberation of a phenolic hydroxyl group and β-hydroxyconiferyl alcohol. The latter alcohol undergoes the Hibbert rearrangement to give the ketones **78—80**. If the condensed units are subjected to acidolysis, the primary reaction products are not released from the macromolecule.

By determining the increase in the phenolic hydroxyl and carbon-substituted methyl group contents effected by the acidolysis, the guaiacylglycerol-β-aryl ether units in Björkman lignin have been estimated to amount to at least one-fourth of the phenylpropane units.

LIGNIN-CARBOHYDRATE BONDS

In 1838 Payen isolated cellulose from wood by dissolving out the incrust material which he assumed to form a physical mixture with cellulose. As wood exhibited marked resistance to the action of cellulose solvents, Erdmann concluded in 1866 that lignin is not free in the wood but is bound to the carbohydrates of wood as a substance which he called glycolignose. Although it has not been possible even yet, a century after Erdmann's report, to solve this question, the results of numerous investigations indicate that lignin is actually combined with carbohydrates.

An idea of the carbohydrates that are bound to lignin can be obtained by an examination of the distribution of the substances in the cell walls of wood. As was already pointed out on p. 15, hemicellulose and lignin are similarly distributed, while the cellulose content is highest in the inner parts of the cell wall and lowest in the outer parts where the major part of the lignin is concentrated. It is thus more probable that the lignin is combined with the hemicellulose components than with cellulose. Also the crystalline structure of cellulose should diminish interaction between lignin and cellulose. However, Wardrop [21] has shown that delignification leads to an increase in the micelle size of cellulose. This can be due to the crystallization of paracrystalline cellulose with which Wardrop assumed the lignin to be associated.

The reason why lignin, with the exception of Brauns' native lignin (p. 73), cannot be dissolved from wood without chemical action can be ascribed to lignin-carbohydrate bonding, but also to the fact that the lignin in wood is polymerized in such a high degree that it must first be broken down before it can be dissolved. It is also possible that the lignin is incrusted by the carbohydrates to such an extent that the latter must be hydrolyzed before the lignin can be dissolved.

If this incrustation theory is correct, it should be possible to extract the lignin from very finely ground wood flour. In fact, Björkman [88, 89] was able to extract more than half of the lignin in spruce wood after grinding the latter in toluene. Björkman employed dioxane first as the extracting solvent and obtained a lignin that contained about 2 per cent carbohydrate. By extraction with dimethyl formamide Lindgren [148] obtained a fraction which he found to consist of a hemicellulose and a lignin-hemicellulose component. As more free lignin was obtained when the dry matter in the dimethyl formamide extract was ground, it is, however, possible that the grinding broke up lignin-hemicellulose bonds.

If the insolubility of protolignin is due to the existence of chemical bonds between lignin and carbohydrates, and since it is highly improbable that these components are united by carbon-carbon bonds, it should be possible to increase the solubility of lignin by subjecting the wood to hydrolysis.

When wood is hydrolyzed in an acid medium, the carbohydrates dissolve as simple sugars, but the lignin remains undissolved. About the effect of highly concentrated hydrochloric acid on wood, Hägglund et al. [149] have stated that when the treatment is of short duration the residue contains carbohydrates and that the acid first dissolves considerable amounts of lignin which later precipitate. It cannot therefore, according to Hägglund, be said that lignin in the state in which it occurs in wood is insoluble in concentrated hydrochloric acid but rather that it is altered by the action of the acid so that it becomes insoluble. The solutions are initially emerald green, which color is probably due to Brauns' native lignin, which like coniferyl aldehyde is colored green by hydrochloric acid.[150, 98]

When wood is hydrolyzed with dilute acid, a part of the carbohydrates becomes soluble in a cuprammonium solution, and the lignin becomes partly soluble in alcohol. It is not, however, necessary to conclude that lignin-carbohydrate bonds are broken in this process for it is possible that the lignin molecule must be broken down, depolymerized, before it dissolves and that this depolymerization is catalyzed by acids. McCarthy et al.[151] have shown that the lignosulfonic acids that dissolve early in the sulfite cooking process are depolymerized later when the cooking continues. This may explain why heating only with acid sulfite solutions can convert hydrochloric acid lignin and periodate lignin into soluble lignosulfonic acids although these lignin preparations are sulfonated but not dissolved by neutral sulfite solutions. Hägglund and Johnson have, however, stressed that their results do not deny the possibility that lignin-carbohydrate bonds are broken in the preparation of hydrochloric acid lignin.[152, 153] Friese et al.,[154, 155] for example, found lignosulfonic acid-carbohydrate complexes in addition to high-molecular lignosulfonic acids in the spent sulfite liquors remaining after the digestion of spruce and beech wood. These authors stated that one third of the lignin in the spent sulfite liquor remaining after pulping of beech wood is bound to carbohydrates.

The alkaline hydrolysis that takes place when wood flour from *Eucalyptus regnans* is repeatedly extracted with 0.5 per cent sodium hydroxide results in a residue whose lignin content does not change as the extraction is continued. Merewether [156] assumed this to be

due either to the breakdown of the lignin-carbohydrate complexes or to simultaneous degradation of alkali-insoluble carbohydrates and lignin to alkali-soluble products.

Brauns' native lignin dissolves readily in cold 4 per cent sodium hydroxide but the main part of wood lignin is much more resistant to alkali. However, the alkali transforms the lignin so that it becomes partly soluble in organic solvents. For example, Harris [157] found that treatment with cold 17.5 per cent sodium hydroxide solution transformed about 70 per cent of maple and aspen lignins and 24 per cent of spruce lignin into products soluble in methanol. He attributed this to the hydrolysis of ester bonds between the lignin and carbohydrates.

THE REACTIONS OF LIGNIN

Sulfonation and Hydrolysis

The sulfonation of lignin during the sulfite cook has been studied primarily by Hägglund and co-workers.[158] According to their two-stage theory the sulfonation first leads to the formation of lignosulfonic acid bound to the wood. A breakdown of lignin-lignin or lignin-carbohydrate bonds takes place in the second stage (p. 59) with the formation of soluble lignosulfonic acid. At the same time the sulfonation proceeds to completion. The sulfonation takes place in both acid and alkaline media, but more rapidly in acid. When spruce sawdust is heated with a 15 per cent sodium bisulfite solution of pH 6 at 135°C and the cooking liquor is replaced every 20 hours, a solid lignosulfonic acid is obtained which contains 0.3 sulfonic acid group per methoxyl group (S/MeO). The degree of sulfonation of the solid lignosulfonic acid cannot be increased in this medium. If the wood is cooked in a sodium bisulfite solution, the sulfonation proceeds much faster and the degree of sulfonation rises to 0.5 S/MeO. At higher acidities such as those employed in industrial sulfite cooking, the rate of sulfonation is even higher, but the hydrolysis also proceeds so rapidly that only small amounts of highly sulfonated solid lignosulfonic acid are found in the pulp. The spent sulfite liquor contains normal lignosulfonic acid with a degree of sulfonation of 0.5 S/MeO. The fact that the degree of sulfonation does not rise above 0.5 S/MeO in the acid industrial cooking process is evidently due to condensation of the lignin.

Whereas practically all of the lignin is dissolved during industrial acid cooking, only about 20 per cent is dissolved when the cook takes place at pH 7. In order to obtain some idea of the rate at which the bonds are hydrolyzed, Lindgren [159, 160] treated spruce wood flour with sulfite solutions of varying pH at 135°C with the results shown in Fig. 4.

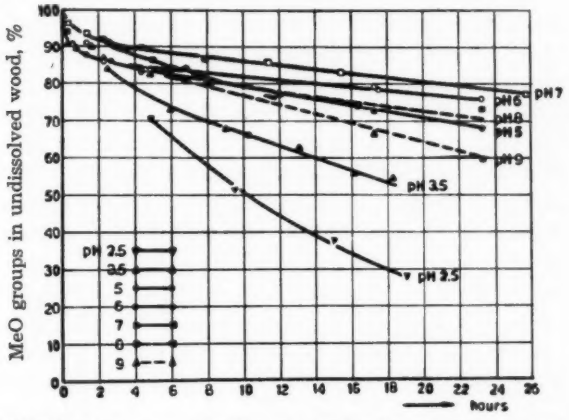


Fig. 4. Delignification as a function of the heating time at 135° C in sulfite solutions of different pH according to Lindgren.[160]

It will be seen from the figure that the lignin dissolves slowly when the wood is heated with neutral sulfite solution but the rate of solution increases when the acidity or alkalinity is increased.

The dissolved lignin is, however, much more highly sulfonated than the undissolved lignin. When Leopold [161] heated extracted spruce wood flour with a sulfite solution of pH 5.3 for 15 hours at 135°C, he found that about 30 per cent of the lignin had become transformed into solid lignosulfonic acid containing about 0.3 S/MeO, while 30 per cent of the lignin was dissolved in the form of low-molecular lignosulfonic acids with degrees of sulfonation (S/MeO) between 0.5 and 0.7. The solid lignosulfonic acids can be dissolved without further sulfonation; Hägglund [162] found that this can be done by heating with mineral acids. Kullgren [163] observed the ability of these sulfonic acids to function as cation exchangers and dissolved the low-sulfonated lignosulfonic acids by replacing the metal ions bound to the sulfonic acid groups with hydrogen ions by treating them with hydrochloric acid. The hydrolysis, which is catalyzed by the hydrogen ions of the sulfonic acid groups, Kullgren effected by heating the material with water.

Condensation

Whereas acid catalyzes the solution of lignin and promotes the sulfonation, it may also have the opposite effect of causing the lignin to condense to larger units. In unfavorable cases leading to a »burnt cook», the condensed lignin can no longer be dissolved. This condensation occurs already during industrial cooking processes as shown, for example, by the fact that the degree of sulfonation does not rise over about 0.5 S/MeO. Also the molecular weights of the lignosulfonic acids found in technical spent sulfite liquor point to an appreciable condensation. Gardon and Mason [164, 165] found that the lignosulfonic acids in spent sulfite liquor may have molecular weights up to 100,000. Thirty per cent of the lignosulfonic acids they studied had a mean molecular weight between 3,700 and 5,000 and an equal amount had a molecular weight between 15,000 and 25,000 (Fig. 5). A similar curve was also obtained by Felicetta, Ahola and McCarthy.[166]

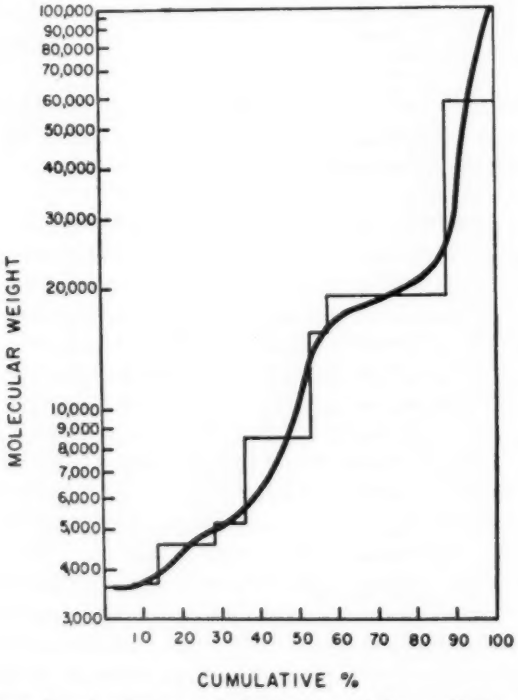
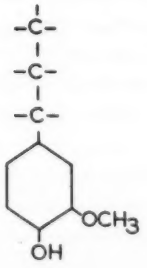


Fig. 5. Integral molecular weight distribution of lignosulfonates according to Gardon and Mason.[164, 165]

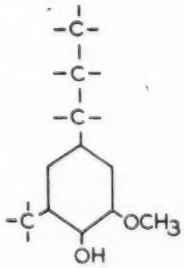
When the sulfonation is continued, a part of the technical lignosulfonic acids may take up more sulfonic acid groups, but a degree of sulfonation corresponding to 1 S/MeO is never attained. Other lignosulfonic acids, especially those produced in a rayon pulp cook, cannot be sulfonated further at all or only to a limited extent.[167] On the other hand, lignosulfonic acids produced under mild conditions are more highly sulfonated than technical lignosulfonic acids. Leopold [168] found that »low-sulfonated lignin» prepared at pH 5 and containing 0.33 S/MeO could be sulfonated to 0.9 S/MeO with a sulfite solution of pH 1.4. It also was possible to obtain a degree of sulfonation of 0.6 S/MeO with a sulfite solution of pH 5.8, although in this case the reaction proceeded very slowly.

By sulfonating spruce wood stepwise at 70 °C by replacing the cooking liquor (11 % SO_2 and 1.45 % NaOH) after each step, Freudenberg, Lautsch and Piazolo [169] obtained 8 soluble lignin fractions. These fractions contained 94—95 per cent of the total lignin and the degree of sulfonation increased from one fraction to the next. The fraction 7 which could still be considered lignin contained 0.96 S/MeO. Determinations showed that the molecular weight of the lignin increased from one fraction to the next. These authors stated that it is not improbable that the increase in molecular weight was due to condensation induced by the acid in the cooking liquor.

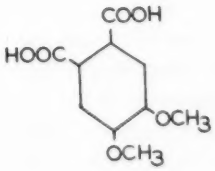
Besides the two phenolic groups **81** and **82**, which are found in all types of lignin preparations, Richtzenhain [132] was able to isolate also metahemipinic acid **(83)** which contains a carbon substituent at position 6. The metahemipinic acid was, however, isolated only from lignin preparations such as hydrochloric acid lignin, alcohol lignin and lignosulfonic acids, i.e. preparations isolated by means of acidic reagents. The yield was only 1 per cent.



(81)



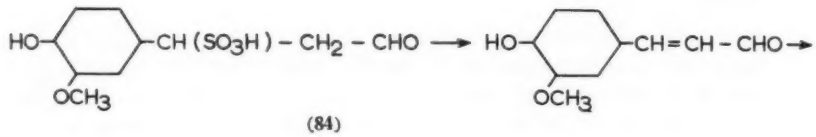
(82)



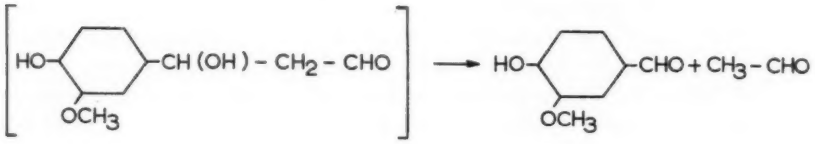
(83)

Richtzenhain concluded that the units condensed at the position 6 are formed by a reaction similar to that by which a lignan is converted into an isolignan (p. 76).

Vanillin is produced when spent sulfite liquors are heated with alkali. This was observed already by Grafe [170] in 1904. Tomlinson and Hibbert [171] obtained vanillin in yields of 4.5—7.1 per cent calculated on the lignosulfonic acids by heating the latter with 19.4 per cent sodium hydroxide solution. They concluded that side chains of the aldol type result from the hydrolysis of sulfonic acid groups and that these side chains are degraded by a reversed aldol condensation:[172]



(84)



Kratzl's [173] observation that also acetaldehyde is formed when lignosulfonic acids are broken down by alkali confirms this assumption.

In contrast to previous observations, Adler and Häggroth [172] showed that vanillin is formed also when lignin is heated with alkali and that the yield of vanillin corresponded to the coniferyl aldehyde group content of the lignin (p. 43).

The lignosulfonic acids, however, give a higher yield of vanillin. Adler and Häggroth believed this to be due to the fact that the lignosulfonic acids contain two different groups that yield vanillin. When the lignosulfonic acids are heated with 0.1 N sodium hydroxide, the vanillin yield corresponded to the coniferyl aldehyde content, but when the alkalinity was increased to 24 per cent sodium hydroxide, more vanillin was produced.

As Hägglund et al.[174, 175] have shown, the vanillin yield increases with the degree of sulfonation. They obtained 2.18 per cent vanillin from a lignosulfonate containing 3.74 per cent sulfur and 6.82 per cent vanillin from a preparation that contained 9.62 per cent sulfur. The sulfonation evidently leads to the formation of a group which on heating with alkali

is eliminated with the formation of vanillin. Since also the yield of acetaldehyde increased with the degree of sulfonation, Adler and Häggroth concluded that this group is derived from a »masked» coniferyl aldehyde group of a type different from that with formula **84**.

The condensation of lignosulfonic acids in acid medium is also indicated by this heating with alkali. Hägglund and Heiwinkel [176] heated at 140°C the spent liquor from a strong pulp cook after they had added to it cooking acid containing 1 per cent calcium oxide and 5.5 per cent sulfur dioxide. When the initially yellow-colored liquor was heated 3.5 hours, it became dark brown and yielded no vanillin on heating with alkali.

Similarly as wood, lignosulfonic acids give a good yield of vanillin when they are oxidized with nitrobenzene and alkali. In this case vanillin is formed also from the unsulfonated part of the lignin. Lautsch and Piazolo [177] fractionated with tar bases the lignosulfonic acids in a spent sulfite liquor from a soft cook and oxidized the fractions with nitrobenzene and alkali. The first four fractions, which comprised about 76 per cent of the total matter, gave a practically constant vanillin yield of about 15 weight per cent. The later (low-molecular) fractions gave higher vanillin yields. This finding Lautsch and Piazolo attributed to the high sulfur contents of the latter fractions.

The vanillin yield and the sulfur content may, however, be two different matters. A high sulfur content does not have to mean a high degree of sulfonation of low-molecular lignin but may be due to the presence in these fractions of other compounds with high sulfur contents. These latter would, of course, lead to lower vanillin yields. The observed large increase in the vanillin yield may be due to the fact that the low-molecular fractions contain other sulfonated aromatic compounds (p. 132).

Leopold [178] oxidized a number of lignans with nitrobenzene and alkali and showed that pinoresinol, lariciresinol and olivil, which contain carbon-oxygen bonds at the α-carbon atom gave respectively 31, 63 and 83 per cent vanillin as calculated from the theoretical maximum yield. It is known that the precipitation technique employed by Lautsch and Piazolo first leads to the precipitation of high-molecular lignosulfonic acids and then to the precipitation of low-molecular acids. The difference in the molecular weights of the high-molecular and low-molecular lignosulfonic acids is, however, so large (p. 62) that one must conclude that the high-molecular aggregates are formed under the influence of the cooking acids after the lignin has been dissolved. If this predominating formation of high-molecular lignosulfonic acids were connected with the formation of carbon-aryl bonds, one would expect that an oxidation of high-molecular lignosulfonic acids with alkaline nitrobenzene

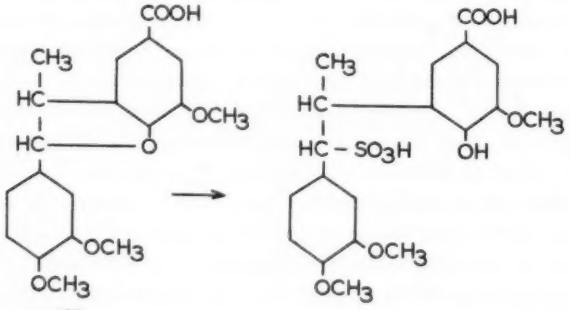
would give lower vanillin yields than the oxidation of the low-molecular acids.

The insensitivity of the vanillin yield to the cooking liquor is shown also by the following experiments of Leopold.[168] He heated »low-sulfonated lignin» (S/MeO = 0.33) with a sulfite liquor of pH 1.4 at 135°C for 24 hours. The sulfonic acid formed contained 0.9 S/MeO, but gave the same vanillin yield (about 32 per cent calculated on the methoxyl content) as the original lignin. Heating with sulfite solutions of pH 3.4 and 5.8 did not result in a lower vanillin yield but only in a higher degree of sulfonation. The heating in acid conditions thus did not lead to carbon substitution at the aromatic nucleus which would have lowered the vanillin yield.

The Mechanism of the Sulfonation Reaction

Klason already suggested that the sulfonation reaction involves the addition of sulfurous acid to double bonds and aldehyde groups. These reactions evidently actually take place in the sulfonation of lignin. Adler et al.[179, 172, 99, 98] showed that lignin contains side chains of the type $-C=C-CHO$ which react with sulfurous acid with the formation of the group $-CH(SO_3H)-CH_2-CH(OH)SO_3H$. However, »true» sulfonic acids are not formed in this way, for treatment with mild alkali liberates sulfite and leaves the original conjugated structure. These sulfonic acid groups hence represent the so-called loosely bound sulfur dioxide (p. 80).

Freudenberg assumed that the sulfonation of lignin involves the rupture of a benzyl aryl ether bond in a heterocyclic ring. Model experiments with acid **85**[180] and similar compounds[181] revealed, however, that the rate of sulfonation of these compounds was low.

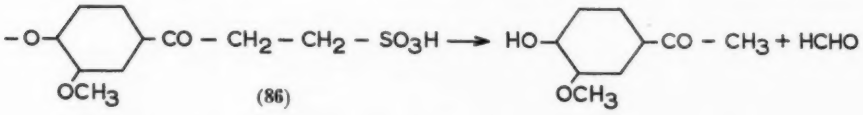


(85)

The rupture of the aryl ether bond leads to the formation of one phenolic hydroxyl group for each sulfonic acid group that is introduced. Experiments later carried out by Freudenberg, Lautsch and Piazolo [169] showed, however, that no increase in the number of phenolic hydroxyl groups occurs in the sulfonation of lignin. They suggested therefore that the sulfonation involves the rupture of a tetrahydrofuran ring of the type present in pinoresinol. Erdtman [182] has, however, shown that neither pinoresinol nor its dimethyl ether can be sulfonated with acid sulfite solutions. Pinoresinol, however, reacts at pH 6—9, whereas its dimethyl ether does not react.[183]

The view that the sulfonation involves a reaction with alcoholic hydroxyl groups was presented by Holmberg [184, 185] who, on the basis of experiments carried out with α-phenylethylcarbinol and diphenylcarbinol, concluded that the groups sulfonated are arylcarbinols or their ethers. Later experiments of Lindgren [160] on model substances such as 4-guaiacylcarbinol and its ethers supported Holmberg's view.

When spent sulfite liquor is made alkaline and heated, acetovanillone is formed in an amount that is up to 10 per cent of the amount of vanillin.[186] This formation of acetovanillone is apparently connected with the formation of formaldehyde.[173, 187] Kratzl [188] concluded from this that lignosulfonic acids may contain a terminal sulfonic acid group and a carbonyl group at the α-position **(86)**.



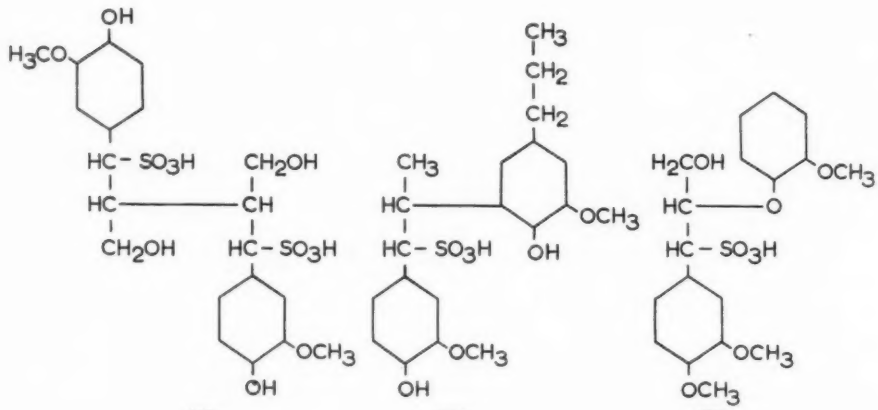
(86)

The question of which groups are sulfonated has been studied also in another way.[189] Erdtman, Lindgren and Pettersson [190] sulfonated »low-sulfonated lignin» and obtained four lignosulfonic acids with sulfur contents varying from 0.27 to 0.63 S/MeO, but in which the sum of the sulfonic acid and hydroxyl groups [(S + OH)/MeO] remained constant. This constant ratio was also found for seven lignosulfonic acids with sulfur contents varying from 0.51 to 0.96 S/MeO which Freudenberg, Lautsch and Piazolo [169] obtained by stepwise sulfonation of spruce wood at 70°C. These results show that at least 50 per cent of the sulfonic acid groups that are introduced in a normal sulfite cook are added to the lignin by the replacement of hydroxyl groups. The mechanism by which the sulfonic acid groups are introduced into the low-sulfonated lignin is not, however, known. Consequently it is not clear whether the sulfonation involves the addition of sulfurous acid or sulfur dioxide

to the lignin, a question of considerable importance when the composition of »lignin» is calculated from the composition of the lignosulfonic acids.

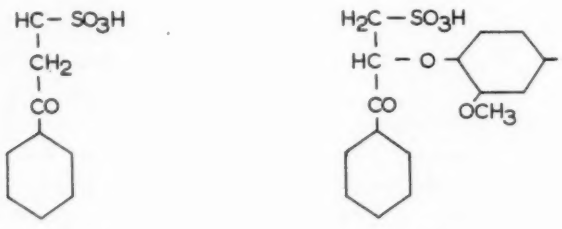
That sulfonic acid groups are bound to phenylpropane units of different types has been demonstrated by Gierer et al.[191, 192] When these authors heated lignosulfonic acids in a mixture of hydrochloric, hydriodic and hypophosphorous acids, they found that one to two thirds of the sulfonic acid groups were eliminated rapidly, but the rest required a much longer time.

Model experiments showed that the rapid phase may possibly involve structures such as **87**, **88** and **76**.



(87) (88) (76)

The slow phase, however, seems to involve either benzylsulfonic acid groups of hitherto unknown types or sulfonic acid groups attached to carbon atoms other than the α-carbon atom. As γ-sulfonic acids like **89** and **90** are very difficult to cleave, Gierer et al. concluded that the lignosulfonic acids may also contain such groupings.

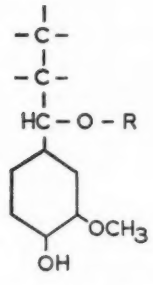


(89) (90)

According to Erdtman [189] lignin contains two types of reactive groups which he designated type A and type B. When groups A are sulfonated, solid insoluble lignosulfonic acids (S/MeO = 0.3) are formed. Groups B react only with acid sulfite solutions. Mikawa [193] and Lindgren [159] showed further that the groups A can be divided into rapidly reacting groups X and slowly reacting groups Z. By acid hydrolysis groups B are converted into groups B' which react with neutral sulfite solutions.[194]

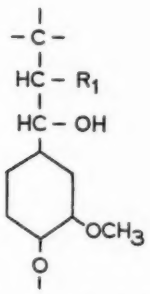
From studies with model substances, Lindgren [160] concluded that the sulfonated groups may have the following structures:

Group X



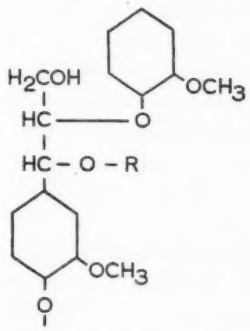
R = H or alkyl

Group Z



R_1 = a small substituent

Groups B and B'



B group : R = alkyl
B' group : R = H

Isolation of Lignosulfonic Acids

Tollens and Lindsey [195] precipitated the lignosulfonic acids from spent sulfite liquor with basic lead acetate. This reagent precipitates about 98.5 per cent of the methoxyl groups in the liquor. The remaining methoxyl groups are more probably bound to the hemicellulose components than to lignin. The lignosulfonic acid precipitated by this method is not, however, pure, for basic lead acetate precipitates also aldonic acids and mannose. Klason [196] also attempted to isolate the lignosulfonic acids and found that approximately half of them could be precipitated with calcium chloride.

Melander [197] found later that 37 per cent of the lignosulfonic acids can be salted out with sodium chloride. The precipitated acids he called

»α-lignin» and the acids remaining in solution »β-lignin». The same division was used also by Klason [198] who applied the name α-lignosulfonic acids to those lignosulfonic acids that are precipitated by 1- or 2-naphthylamine and the name β-lignosulfonic acids to those that are not precipitated.

This division of the lignosulfonic acids was the reason for Klason's belief that spruce wood contains two kinds of lignin, α- or acrolein-lignin and β- or carboxyl-lignin. These latter designations were due to the occurrence of acrolein and carboxyl groupings in the α- and β-lignosulfonic acid preparations.

The precipitation of the lignosulfonic acids from spent sulfite liquor is not, however, always the same but depends on the conditions of the cook. Thus, for example, a larger precipitate is obtained from the spent liquor from a rayon cook than from the liquor from a cook of strong pulp. Lignosulfonic acids that have been purified by dialysis can be precipitated quantitatively, but the lignosulfonic acids in a liquor produced under mild conditions can be precipitated only partly. For this reason Hägglund [199] did not accept the view that β-lignin occurs initially in the wood but stated that the deviating properties of α- and β-lignosulfonic acids are a consequence of the effect of the cooking liquor.

In order to obtain some insight into the nature of the β-lignosulfonic acids, Hägglund [199] precipitated the α-lignosulfonic acids from a spent liquor with 1-naphthylamine and precipitated the acids remaining in the liquor as their barium salts by adding alcohol. From the latter precipitate he removed the carboxylic acids by treatment with the calculated amount of sulfuric acid. The β-lignosulfonic acids thus isolated had an exceptionally high sulfur content and a low methoxyl content. The acids reduced Fehling's solution more effectively than the α-lignosulfonic acids. Hägglund was not able to detect any carboxyl groups in the β-lignosulfonic acids. Analyses of the barium salts of the β-lignosulfonic acids gave the following results:

$$S = 7.35\ \%,\ Ba = 15.80\ \%,\ CH_3O = 5.02\ \%,\ \text{Eq. wt.} = 435$$
$$\text{Copper number} = 18\text{—}19 \quad \text{Ratio } S{:}CH_3O = 1{:}0.7$$

For comparison, the analytical data for barium lignosulfonates obtained by salting out give,[200]

$$S = 6.2\ \%,\ Ba = 11.5\ \%,\ CH_3O = 11.6\ \%$$

The fact that β-lignosulfonic acids have a definitely lower methoxyl content than α-lignosulfonic acids Hägglund assumed to be due to the elimination of methoxyl groups during the cooking process.

When primary amines are employed as precipitants, the amine lignosulfonate formed cannot be completely decomposed with alkali. The reason for this is probably that the carbonyl groups in the lignosulfonic acids form Schiff's bases with the precipitant.

$$R - NH_2 + O = C\begin{matrix}\nearrow\\\searrow\end{matrix} \longrightarrow R - N = C\begin{matrix}\nearrow\\\searrow\end{matrix} + H_2O$$

To avoid this, Erdtman [201, 57, 202] employed as precipitant the tertiary amine 4,4-bis-(dimethylamino)-diphenylmethane. This reagent precipitates 60—90 per cent of the lignosulfonic acids in spent liquors. These acids behave like typical α-lignosulfonic acids; for example, they are almost completely undialysable. Erdtman precipitated a second fraction from the mother liquor with brucine and a third fraction with basic lead acetate. From these three fractions he removed the carboxylic acids by treatment with sulfuric acid.

The isolated fractions varied considerably in composition. The α-lignosulfonic acids contained about 0.5 S/MeO. The low-molecular fractions, like Hägglund's β-lignosulfonic acids, had high sulfur and low methoxyl contents. The lignosulfonic acids of high molecular weight had a low copper number which did not rise appreciably on hydrolysis. From this Erdtman concluded that they did not contain glucosidically bound carbohydrates.

The low-molecular lignosulfonic acids, on the other hand, had a higher copper number which increased further on hydrolysis. According to Erdtman et al.[57, 58] this means that they may be present as mixtures with other sulfonic acids, such as polysaccharide sulfonic acids, or are glucosidically combined with the latter. This would explain why they differed so much in composition from the high-molecular lignosulfonic acids. In this respect their higher oxygen and lower methoxyl contents are noteworthy.

A similar change in the composition of lignosulfonic acids with decreasing molecular weight was noted by Gardon and Mason [164, 165] who divided the lignosulfonic acids in a spent sulfite liquor into eight fractions by dialysis and ultracentrifugation (Tables VII and VIII).

Table VII

Analytical Data for Sodium Lignosulfonate Fractions According to Gardon and Mason [165]

Fraction No.	% of Total Original Solids	% of Total of Fractions	% Methoxyl	% S	% Phenolic Hydroxyl*	Reducing Power, % Eq. Glucose	Neutralization Eq.Wt.	Acid Groups	Sulfur S / MeO	Mol. Wt.
1	7.3	11.87	10.45	5.22	0.945	3.56	599	1.03	0.53	58,000
2	18.7	30.41	10.40	5.58	0.739	3.36	570	1.01	0.56	19,200
3	2.8	4.55	10.41	5.40	0.780	3.35	585	1.01	0.55	15,500
4	10.2	16.58	9.82	5.98	0.870	3.79	535	1.00	0.64	8,450
5	4.8	7.80	8.80	6.37	0.770	7.18	450	1.11	0.81	4,770
6	9.5	15.45	7.24	6.25	0.696	9.20	375	1.36	0.89	4,350
7	3.4	5.53	4.06	5.55	0.672	9.42	289	2.00	1.46	3,700
8	4.8	7.80	2.25	5.02	0.530	8.47	227	2.81	2.38	3,650
Total	61.5	100								

* Calculated from the differential ultraviolet extinction coefficient.

Table VIII

Characteristic Data from Ultraviolet Absorption Spectra of Sodium Lignosulfonate Fractions According to Gardon and Mason [165]

	pH 6						pH 12			Differential Spectrum	
Fraction No.	ε 280, cm^{-1} g^{-1} liter	Absorbance at 280 mμ per methoxyl-bearing unit	λ_{max}, mμ	λ_{min}, mμ	$\varepsilon_{max}/\varepsilon_{min}$,	$\varepsilon_{245\,m\mu}/\varepsilon_{260\,m\mu}$	λ_{max}, mμ	λ_{min}, mμ	$\varepsilon_{max}/\varepsilon_{min}$,	λ_{max}, mμ	$\Delta\varepsilon_{max}$, cm^{-1} g^{-1} liter
1	13.9	4200	282	263	1.32	2.28	282	266	1.13	300	2.28
2	13.3	4090	283	263	1.39	2.28	281	267	1.13	298	1.78
3	13.5	4150	282	262	1.38	2.23	281	267	1.12	301	1.88
4	12.4	4020	282	262	1.29	2.20	281	268	1.07	298	2.10
5	11.1	4100	283	263	1.39	2.15	281	268	1.09	299	1.86
6	8.8	3900	283	262	1.38	2.05	282	270	1.10	300	1.68
7	5.8	4200	277	261	1.34	1.89	281	270	1.16	299	1.62
8	4.2	6360	277	258	1.30	1.59	282	273	1.09	297	1.28

Gardon and Mason concluded from their analyses that the fractions 1—4 consisted of pure lignosulfonates, for the fractions contained no other acid groups than the sulfonic acid group, and their methoxyl contents and absorptivities at 280 mμ were high. Their reducing power, on the other hand, was low.

As in the case of Erdtman's low-molecular lignosulfonic acids, they were unable to decide whether the fractions 5—8 were composed only of pure lignosulfonates. They contained also other acid groups than the sulfonic acid group, had low methoxyl contents, and were strong reductants.

Freudenberg, Lautsch and Piazolo [169] also found other acid groups than sulfonic acid groups in the various lignosulfonic acid fractions they isolated from spent sulfite liquors. They assumed the other acid groups to be carboxyl groups.

The light absorption in the ultraviolet range indicates the chemical similarity of the lignosulfonate fractions isolated by Gardon and Mason. The absorptivity (ε) at 280 mμ calculated per methoxyl-binding unit was the same for all fractions except the last. This was first noted by Aulin-Erdtman [203] who found an α- and a β-lignosulfonic acid fraction to have the same absorptivity (ε) although the concentration of the latter fraction was four times that of the former. Aulin-Erdtman drew the conclusion that the β-lignosulfonic acid contained a methoxyl-free component that did not absorb ultraviolet light. She was unable to determine with her spectrophotometric methods whether the latter component was bound to lignin or not.

BRAUNS' NATIVE LIGNIN AND THE LIGNANS

When Brauns [204] extracted wood flour with ethyl alcohol, he obtained an extract which amounted to 8—10 per cent of the lignin originally present in the wood. Adler and Gierer,[205] however, were able to extract only 1—2 per cent of the lignin. This Brauns' »native» lignin exhibits many of the properties of lignin. It is, for example, sulfonated by a sulfite cooking liquor. It differs from the main part of the lignin by its higher phenolic hydroxyl group content (p. 43).

Whereas the aromatic constituents of wood and also their sulfonic

acid derivatives give very similar ultraviolet spectra in acid or neutral solution, their spectra in alkaline solution differ greatly. A shift of the absorption maximum at 280 mμ to longer wave lengths occurs in the spectra of Brauns' native lignin and its sulfonated form when the solutions are made alkaline; in addition, a new absorption band appears at about 250 mμ (Fig. 6).

However, the absorption maxima at 280 mμ in the spectra of the main part of the lignin and of the lignosulfonic acids in spent sulfite liquor do not shift when the medium is made alkaline (Fig. 7).

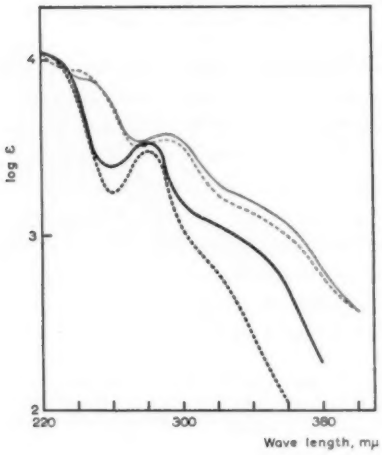


Fig. 6. Ultraviolet absorption spectra* of Brauns' native lignin (BNL) and sulfonated Brauns' native lignin (BNLS) (*Picea excelsa*) according to Aulin-Erdtman.[206]

—— BNL in 95 % EtOH
—— BNL in N ethanolic KOH
--- BNLS in water + 0.75 % of cooking acid
--- BNLS in N aqueous NaOH

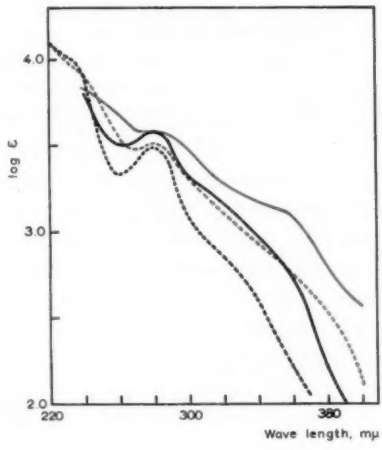


Fig. 7. Ultraviolet absorption spectra* of Björkman lignin (MWL) (*Picea excelsa*) according to Björkman [89] and lignosulfonic acid (LSA) (*Picea excelsa*) according to Aulin-Erdtman.[207]

—— MWL in neutral methyl cellosolve
—— MWL in alkaline methyl cellosolve**
--- LSA in water, pH 7
--- LSA in water, pH 13

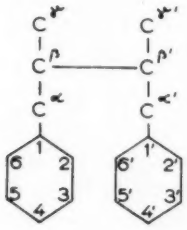
* The letter ε denotes molar absorptivity ($l \cdot [CH_3O]^{-1} \cdot cm^{-1}$).

** Calculated with the aid of the $\Delta\varepsilon$-curve for MWL.

Freudenberg and Knof [208] isolated a lignin similar to Brauns' native lignin by extracting spruce wood (*Picea excelsa*) with acetone-water (17:3). The extract, which amounted to 1.6 per cent of the weight of the wood, comprised up to 31 per cent of phenol-free matter, up to 31 per cent of a lignin-like component and up to 38 per cent lignans.

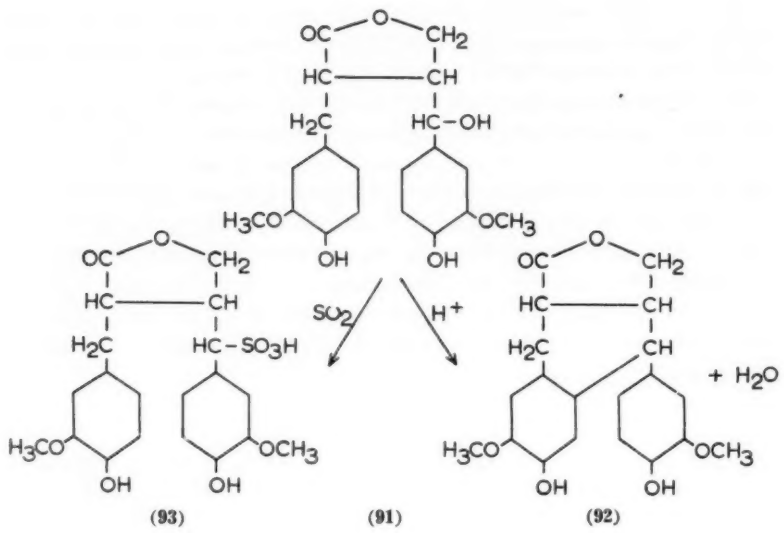
The lignans are a group of naturally occurring compounds that consist of double phenylpropane units. They have been found in all parts of plants, sometimes as glucosides, but their function in the plant is not known. In contrast to lignin, most lignans are optically active.[209]

Erdtman [209] assumed that the lignans are formed in principally the same way as lignin, i.e. by dehydrogenation of C_6C_3 precursors and β,β-coupling of the formed radicals (p. 37). The resulting structure is shown below.



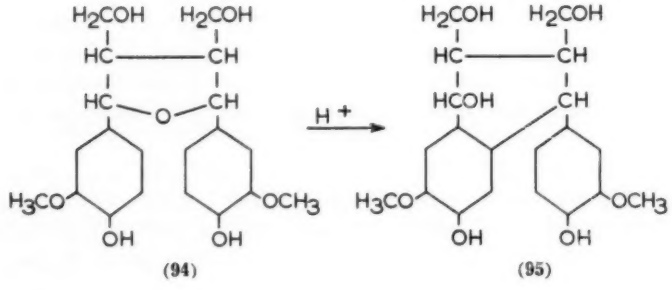
Besides the carbon-carbon bond between the β- and the β'-carbon atoms, which is common to all lignans, they may also contain carbon-carbon bonds linking carbon atom 2 to the α'-carbon atom, as in α-conidendrin **(92)**. Lignans also frequently have a γ,γ'-ester linkage forming a γ-lactone ring. The phenylpropane units may be joined also by ether linkages.

Some lignans, like hydroxymatairesinol **(91)** and liovil **(96)**, have hydroxyl groups at the carbon atoms adjacent to the aromatic nuclei and may therefore become sulfonated **(93)** at these points during a sulfite cook. These hydroxyl groups readily participate in condensation reactions. Freudenberg and Knof [208] showed that hydroxymatairesinol and allo-hydroxymatairesinol are precursors of α-conidendrin and are converted into the latter by treatment with cold formic acid.



(93) (91) (92)

Olivil **(94)**, which contains an α,α'-ether linkage, is converted to isoolivil **(95)** almost quantitatively by boiling dilute acetic or formic acid.[210] This conversion leads to the formation of a hydroxyl group at the α-carbon atom.



(94) (95)

Ultraviolet spectra of α-conidendrin are shown in Fig. 8. As seen from the figure, the absorption maximum at 280 mμ in the ultraviolet

spectrum recorded in neutral solution is shifted to longer wave lengths in alkaline solution, similarly as in the case of Brauns' native lignin. A new maximum also appears at 245 mμ.

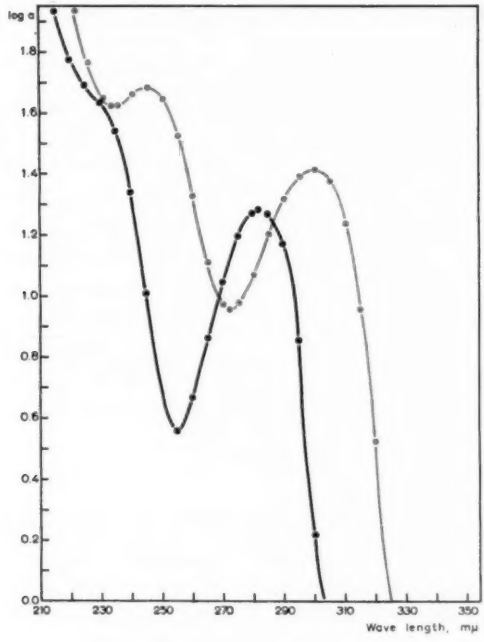
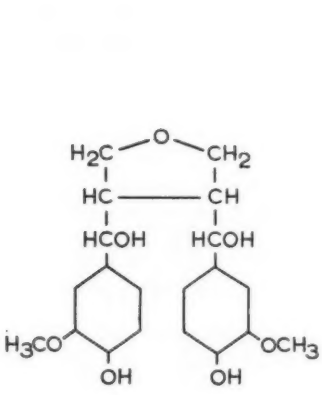


Fig. 8. Ultraviolet absorption spectra* of α-conidendrin.

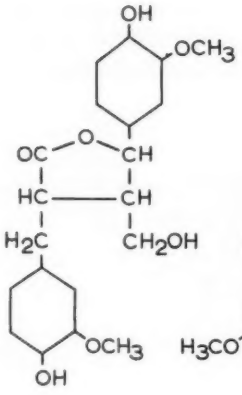
— Dissolved in ethanol and diluted with water.
– – Dissolved in water made alkaline with NaOH to pH 13.

* The letter a denotes absorptivity ($l \cdot g^{-1} \cdot cm^{-1}$).

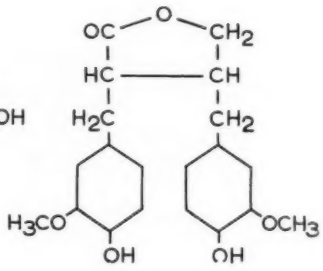
Freudenberg and Knof [208] isolated from spruce wood the following compounds, which together amounted to about 0.4 per cent of the wood:



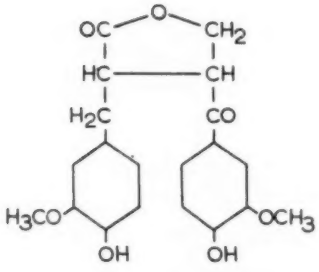
(96)



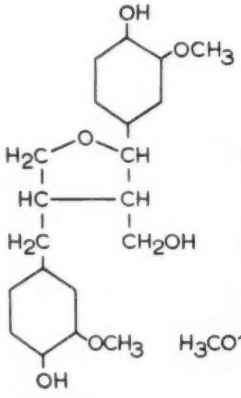
(97)



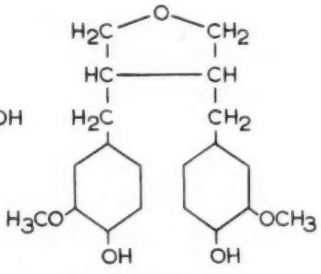
(98)



(99)



(100)



(101)

It is seen from Table IX that hydroxymatairesinol and allo-hydroxymatairesinol are the most important lignans in spruce wood. Their structures have not yet been fully clarified. Freudenberg and Knof concluded that they are either isomers like **(91)** and **(97)** or carbinol group epimers. The reason why these lignans have not been isolated from spent sulfite liquor is probably that they form sulfonic acids that are difficult to separate from the lignosulfonic acids.

α-Conidendrin or »sulfite waste liquor lactone», on the other hand,

Table IX

Lignans and other Aromatic Compounds Isolated from Spruce Wood According to Freudenberg and Knof [208]

	Estimated percentage in wood
(—) - Hydroxymatairesinol, **(91)** or **(97)**	0.160 %
(—) - Allo-hydroxymatairesinol, **(91)** or **(97)**	0.096 %
(—) - α-Conidendrin, **(92)**	0.048 %
(+) - Pinoresinol, **(25)**	0.019 %
(—) - Liovil, **(96)**	0.019 %
(—) - Matairesinol, **(98)**	0.016 %
(+) - Oxomatairesinol, **(99)**	0.016 %
(—) - Lariciresinol, **(100)**	0.016 %
Coniferyl aldehyde, **(27)**	0.008 %
Vanillin, **(60)**	0.002 %
3,4-Divanillyl-tetrahydrofuran, **(101)**	0.002 %

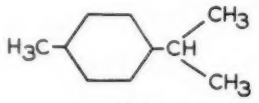
is one of the best known of the lignans. As revealed by the latter name, α-conidendrin is found in spent sulfite liquor. It is a colorless compound melting at 255—256°C. Another crystalline modification melts at 238°C.[211] Holmberg [212, 213] isolated 0.2—0.3 g of the compound from one liter of spruce spent sulfite liquor (*Picea excelsa*) and Hintikka [214] found that α-conidendrin could be detected already at an early stage of the cook and that its amount rises to a maximum and then decreases toward the end of the cook.

OTHER COMPOUNDS AND LOOSELY BOUND SULFUR DIOXIDE IN SPENT SULFITE LIQUOR

p-CYMENE

Varying amounts of p-cymene **(102)** are formed during the sulfite

cook. According to Lassenius [215] the yield is usually about 0.2 kilogram



(102)

per ton of pulp, but up to one kg of p-cymene per ton of pulp has been obtained from fresh wood.[216]

According to Routala and Pohjola,[217] p-cymene is formed from pinene as follows:

Pinene ⟶ Terpineol ⟶ Terpinene ⟶ p-Cymene

The reaction mechanism is the same as that of aldonic acid formation:

$$2\ C_{10}H_{16} + 2\ HSO_3^- \longrightarrow 2\ C_{10}H_{14} + S_2O_3^{2-} + 3\ H_2O$$

FORMALDEHYDE

Adler [65] demonstrated the existence of formaldehyde in spent sulfite liquor. He found a strong pulp spent liquor to contain 0.18 g of formaldehyde per liter and a rayon pulp spent liquor to contain 0.54 g/l.

LOOSELY BOUND SULFUR DIOXIDE

Spent sulfite liquor contains besides a small amount of sulfur dioxide that is directly titratable with iodine (which must not be confused with »free sulfur dioxide») also so-called loosely bound sulfur dioxide. The latter is liberated by alkali and can then be titrated iodometrically. In rayon pulp spent liquors the content of loosely bound sulfur dioxide is between 1 and 3 g/l, whereas in strong pulp spent liquors it may be as high as 6 g/l.[65]

Adler [218] has confirmed the opinion that the loosely bound sulfur dioxide occurs in aldehyde-bisulfite addition compounds (α-hydroxy-sulfonic acids). The following equilibria thus prevail in spent sulfite liquors:

$$(\text{I}) \quad R - CH(OH)SO_3^- \rightleftharpoons R - CHO + HSO_3^-$$

$$(\text{II}) \quad SO_2 + OH^- \rightleftharpoons HSO_3^- \rightleftharpoons H^+ + SO_3^{2-}$$

$$(\text{III}) \quad SO_2 + H_2O \rightleftharpoons H_2SO_3$$

In highly acid solutions such as rayon pulp spent liquors the equilibrium II is shifted to the left and the greater part of the loosely bound sulfur dioxide escapes. The less acid strong pulp spent liquors therefore contain more loosely bound sulfur dioxide. When the solution is made alkaline, the equilibrium shifts to the right and the bisulfite addition compounds are broken down.

Adler [218] found that the liquor contains a group of aldehyde-bisulfite addition compounds which can be decomposed by neutralizing with chalk to pH 5—6, and another group that is not decomposed unless the pH is over 7.

Adler [65] found also that the compounds that add sulfur dioxide are primarily lignosulfonic acids and three volatile aldehydes formaldehyde, methylglyoxal and furfural; the bisulfite addition compounds of the latter two aldehydes are not decomposed by chalk. In addition there occurs in strong pulp spent liquor a carbonyl compound of unknown structure which can be dialysed through cellophane and which has a great tendency to bind sulfur dioxide. The lesser importance of monosaccharide-bisulfite addition compounds has been demonstrated independently by Adler [65] and Sundman.[219]

In one of the rayon pulp spent liquors examined by Adler the three volatile aldehydes bound approximately 90 per cent of the loosely bound sulfur dioxide. During rayon pulp digestion the lignosulfonic acid-bisulfite addition compounds are decomposed to a large extent. Also the carbonyl properties of the lignosulfonic acids are partly destroyed. For this reason the lignosulfonic acids in rayon pulp spent liquors bind only insignificant amounts of sulfur dioxide.

In strong pulp spent liquors the reverse is true. The volatile aldehydes bind only a lesser proportion of the loosely bound sulfur dioxide, the greater part being bound by nonvolatile aldehydes, mainly by the coniferylaldehyde units in the lignosulfonic acids.

THE INVESTIGATION OF A SPENT SULFITE LIQUOR

ION EXCLUSION

GENERAL

Organic ion exchange resins consist of insoluble cross-linked, tridimensional polymers into which various ionizing groups have been introduced. In cation exchange resins the latter groups are either sulfonic acid or carboxyl groups, whereas in anion exchange resins the groups are quaternary ammonium or amino groups.

Both the strongly acid cation and the strongly basic anion exchange resins are usually prepared by copolymerizing styrene and divinylbenzene which leads to cross-linking in the resin. The cation exchange resin employed in the present study, Dowex-50, X-2, H^+, 100/200 mesh, is a nuclear sulfonated polymer of the above type. Dowex-50 is the trade mark* for a group of strongly acid cation exchange resins. The designation X-2 means that 2 per cent divinylbenzene has been employed in the manufacture of this particular resin and thus indicates the degree of cross-linking or porosity of the resin. Dowex-50, X-2, is a highly porous resin. The symbol H^+ means that the ion exchange resin is in the hydrogen (acid) form. The grain size is indicated by giving the mesh between which the grains lie. Expressed in metric units, 100/200 mesh means that the diameters of the grains vary from 0.149 to 0.074 mm.[220]

When they come into contact with polar solvents such as water, ion exchange resins bind the solvent and swell. The swollen form of Dowex-50, X-2, contains 76—86 per cent water, whereas the less porous Dowex-50, X-8, contains 45—56 per cent water.[220] The total volume

---

* Registered by Dow Chemical Co., Midland, Mich., U.S.A.

of a column of ion exchange resin and water may be taken to consist of three parts:

$$V_t = V_o + V_i + V_r$$

V_t is the total volume of the ion exchange column, V_o the volume between the resin particles, V_i the volume of water occluded within the resin particles and V_r the volume of the resin itself.

When an ion exchange resin is added to an electrolyte solution and the exchangeable ion is the same in both, the concentration (C_i) of the ion in the water within the resin particles (V_i) is lower than its concentration (C_o) in the solution between the particles (V_o). This distribution is expressed by means of the distribution coefficient:

$$K_d = \frac{C_i}{C_o}$$

The value of the distribution coefficient for the system Dowex-50, X-8, H^+ — hydrochloric acid is approximately 0.1 when C_o does not exceed 1 molal. This uneven distribution of electrolytes is typical for strongly ionized ion exchange resins and has led to the term ion exclusion.[221] Nonelectrolytes are excluded from the interior of the resin particles in a lesser degree and can therefore be separated from electrolytes by ion exclusion. If, for example, a solution containing two compounds A and B with distribution coefficients 0 and 1, respectively, is passed through an ion exchange column, A will appear in the effluent when the volume V_o of solvent has been replaced by new solvent. The component B appears in the effluent when the volume of the latter is $V_o + V_i$. The general expression for the effluent volume V when a dissolved compound with distribution coefficient K_d emerges from the column is

$$V = V_o + K_d V_i$$

The distribution of dissolved compounds between the resin and the intermediate phase is influenced also by their molecular size. In the case of high-molecular compounds the ion exchange resin functions as a molecular filter. Adsorption to the ion exchange resin is a process that also promotes the separation of solutes.

EARLIER WORK ON THE FRACTIONATION OF SPENT SULFITE LIQUORS BY ION EXCLUSION

The first attempts to separate the acids in spent sulfite liquor by ion exclusion were made by Samuelson [222] in 1956. He found that no irreversible absorption of the components of spent sulfite liquor took place and that a partial separation of strong and weak acids in the spent sulfite liquor could be effected by the method. The separation of strong and weak acids in spent sulfite liquor by ion exclusion was studied also by Hartler.[223]

In 1957 Shaw [224,225] described a method he had developed for the determination of the sugars in the spent liquor from a calcium base sulfite cook. He passed a sample of the spent liquor through a cation exchange resin in the calcium form. The high-molecular lignosulfonic acids were separated from the sugars and low-molecular acids which passed less rapidly through the column and emerged together. The latter acids were removed from the sugar solution by means of an anion exchange resin. The separation of lignosulfonic acids from the sugars in spent sulfite liquor by ion exclusion was studied further by Felicetta, Lung and McCarthy [226] who were able to divide the compounds in a spent liquor into two fractions, one containing mainly lignosulfonic acids and the other primarily sugars.

As it seemed that the ion exclusion method could be developed further to effect a good separation of the compounds in spent sulfite liquor, experiments were initiated by the present writer which gave the results that are described below.

THE FRACTIONATION OF THE ORGANIC SOLUTES IN A SPENT SULFITE LIQUOR BY ION EXCLUSION

THE PRELIMINARY FRACTIONATION OF THE SPENT SULFITE LIQUOR

A spent liquor from an industrial sulfite cook of spruce wood (*Picea excelsa*) that yielded a strong pulp was treated as shown in Fig. 9 in order to isolate the nonvolatile organic components, which were finally collected in solution L_4.

The salts in four hundred milliliters of the spent sulfite liquor, so-

lution L_1, were converted into the acid form by passing the liquor through Dowex-50, X-8, H^+, 50/100 mesh, that had been treated in the same manner as the ion exchange resin employed for ion exclusion (p. 91).

Volatile substances were removed from the acid effluent at 45°C with a stream of nitrogen during 30 hours. The compounds removed,

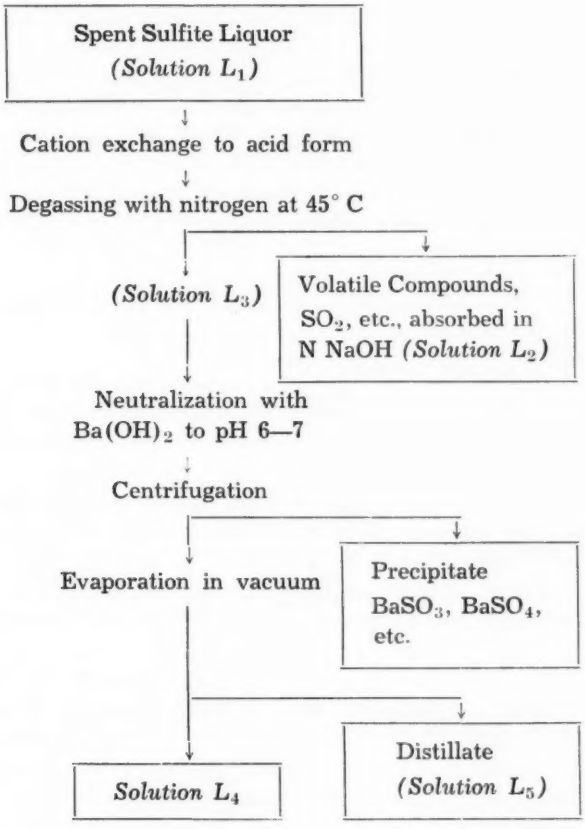


Fig. 9. Preliminary fractionation of the spent sulfite liquor.

primarily sulfur dioxide, were absorbed in N sodium hydroxide. The resulting solution L_2, which was pale yellow in color, was diluted to 200 ml with distilled water.

The solution L_3 from which the cations and volatile compounds had been removed was diluted to one liter. Five hundred milliliters of the resulting solution were neutralized to pH 6—7 with barium hydroxide solution. The small precipitate, which was not investigated further, but which evidently consisted of barium sulfite and barium sulfate, was removed by centrifugation. The clear supernatant was evaporated on a 40-degree water bath under reduced pressure to 100 ml, whereupon solution L_4 was obtained. The distillate, solution L_5, was about 900 ml in volume.

The following table (X) gives the analytical data for the total sulfur, loosely bound sulfur dioxide and directly titratable sulfur dioxide in the solutions L_1, L_2 and L_4. The total sulfur was determined gravimetrically after oxidation with nitric acid by a modification of the TAPPI method 629-m 53. The loosely bound and directly titratable sulfur dioxide were determined iodometrically by the TAPPI method 629-m 48.

Table X

Distribution of Sulfur during the Preliminary Fractionation of the Spent Sulfite Liquor

Solution	Total S		Directly titratable SO_2		Loosely bound SO_2	
	as S, g/l	% of total S in L_1	as S, g/l	% of total S in L_1	as S, g/l	% of total S in L_1
L_1	9.69	100	0.10	1.1	2.51	25.9
L_2	—	—	3.65	18.8	—	—
L_4	16.10	83.1	0.00	0.0	1.58	8.1

The solution L_4 thus contained no directly titratable sulfur dioxide. Of the sulfur present in 200 ml of the original spent sulfite liquor (solution L_1), 83.1 per cent was found in solution L_4. The rest of the sulfur, with the exception of the discarded precipitate, was present as directly titratable sulfur dioxide in solution L_2.

It was expected that at most $1.1 + 25.9 = 27.0$ per cent of the total sulfur in solution L_1 would be removed in the treatment of this solution.

Of the total sulfur in solution L_1, 18.8 per cent was found as directly titratable sulfur dioxide in solution L_2, whereas 8.1 per cent remained as loosely bound sulfur dioxide in solution L_1. The latter two percentages total 26.9 per cent.

Of the total sulfur content of solution L_4, 9.8 per cent ($= 100 \cdot 8.1/83.1$) was loosely bound sulfur dioxide. Of the loosely bound sulfur dioxide in solution L_1, 31.3 per cent ($= 100 \cdot 8.1/25.9$) was thus not liberated on acidifying the solution.

In the customary iodometric determination of the directly titratable sulfur dioxide in spent sulfite liquor, the presence of thiosulfate is disregarded. According to Samuelson et al. [227] spent sulfite liquor may contain thiosulfate corresponding to 0.1—0.5 g S/l which reacts, like sulfite, with iodine.

$$SO_3^{2-} + J_2 + H_2O \rightarrow SO_4^{2-} + 2\ J^- + 2\ H^+$$

$$2\ S_2O_3^{2-} + J_2 \rightarrow S_4O_6^{2-} + 2\ J^-$$

According to Table X the spent sulfite liquor L_1 contained 100 mg of sulfur per liter as »directly titratable SO_2». This amount is equivalent to $100/32.1 = 3.12$ millimoles of iodine per liter. Assuming that the liquor contained 200 mg S/l as thiosulfate, the consumption of iodine due to thiosulfate should have been $200/(32.1 \cdot 4) = 1.56$ millimoles per liter. The »true» titratable sulfur dioxide therefore corresponded to only $(3.12—1.56) \cdot 32.1 = 50$ mg S/l. It is thus obvious that the true directly titratable sulfur dioxide in spent sulfite liquor cannot be determined iodometrically without a separate determination of the thiosulfate content.

Other analytical data for solutions L_1—L_5 are shown in Table XI.

The acid contents of solutions L_1 and L_4 were determined by passing samples of the solutions through Dowex-50, X-8, H^+, 50/100 mesh, and titrating the effluents in tared vessels potentiometrically with 0.02 N sodium hydroxide to pH 8.0 in a nitrogen atmosphere. Illustrative titration curves are shown in Fig. 10.

The dry matter content was determined by evaporating the titrated solutions to constant weight at 60° C. The dry matter contents hence refer to samples (of L_1 and L_4) in which the cations had been replaced by sodium.

The methoxyl contents of these evaporation residues were determined by the micro Zeisel method.[228]

Table XI

Analytical Data for Solutions L_1—L_5

Solution	L_1	L_2	L_3	L_4	L_5
Volume, ml	400	200	1000	2 × 100	2 × 900
Dry matter (sodium salts), mg/ml	189.3 186.5 186.8 188.0 } 187.7			350.5 346.0 348.5 344.5 } 347.4	
Dry matter as a percentage of the amount in L_1	100			92.5	
Methoxyl content of the dry matter (sodium salts), %	5.89;5.81 6.12;6.43 5.98;5.74 } 6.00			6.68 6.26 } 6.47	
Methoxyl groups as a percentage of the amount in L_1	100			99.8	
Acidity, mequiv./ml	0.527 0.526 0.530 0.531 0.525 } 0.528			0.796 0.800 0.788 0.796 } 0.795	
Equivalents of acid as a percentage of the equivalents in L_1	100			75.3	
Absorbance at 280 mμ	1244	4.0	489.5	2362	0.3
Absorbance at 280 mμ as a percentage of the absorbance of solution L_1	100	0.2	98.4	94.9	0.1
Absorbance at 380 mμ after reaction with o-aminodiphenyl	130.6 130.0 } 130.3	3.84 3.91 } 3.88	52.8 51.0 } 51.9	254.4 252.6 } 253.6	0.03 0.07 } 0.05
Absorbance at 380 mμ after reaction with o-aminodiphenyl as a percentage of the absorbance of solution L_1	100	1.5	99.6	97.3	0.2

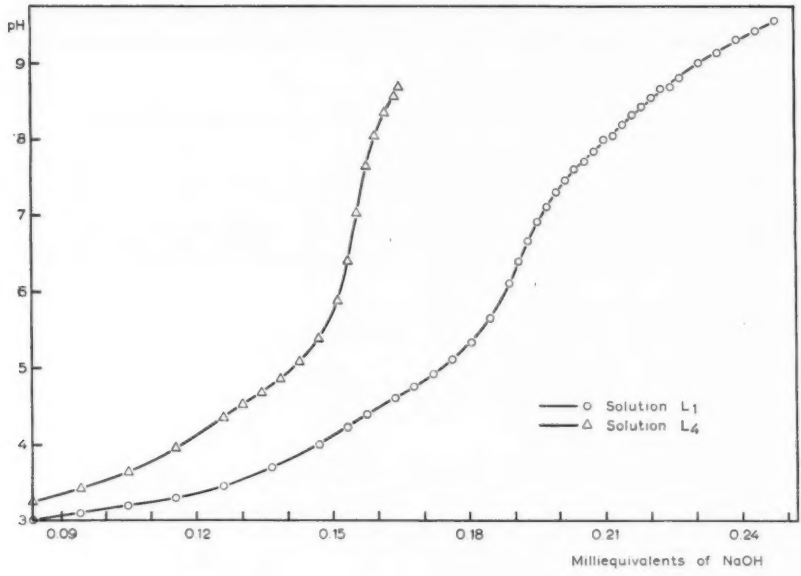


Fig. 10. Potentiometric titration curves for solutions L_1 and L_4.

Solution L_1 was passed through a cation exchange resin and diluted to a fiftyfold volume and 20 ml of the resulting solution titrated with 0.0210 N NaOH. Solution L_4 was passed through a cation exchange resin and diluted to a fiftyfold volume and 10 ml of the resulting solution was titrated with 0.0210 N NaOH.

The absorbances at 280 mμ of the solutions L_1, L_2, L_4 and L_5 were measured after suitable dilution in quartz cuvettes with an optical path of 1.000 cm in a Beckman spectrophotometer model DU. Solution L_2 was neutralized beforehand with hydrochloric acid. The ultraviolet spectra of the various solutions are shown in Fig. 11.

The absorbances at 380 mμ were measured after one milliliter of each solution had been suitably diluted, 5 ml of a solution composed of 0.4 g of o-aminodiphenyl, 100 ml of glacial acetic acid and 20 ml of water had been added, and the mixture had been heated one hour on a boiling water bath in 20-ml test tubes closed by rubber stoppers covered with aluminum foil. The absorbance was measured against a solution prepared in the same way, but in which water was substituted for the test solution.

It is seen from Table XI that all the methoxyl groups in the spent sulfite liquor except those bound to the volatile neutral components,

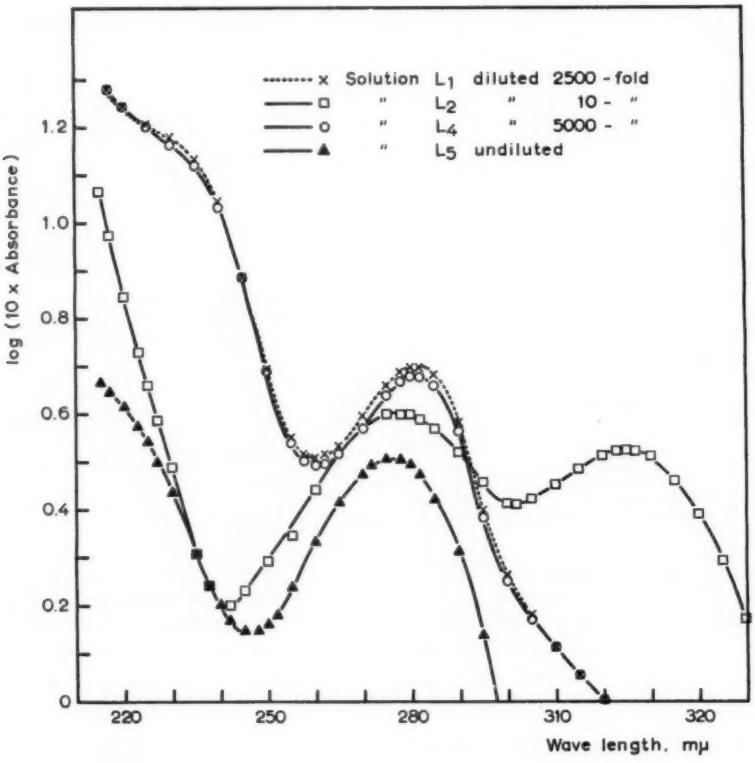


Fig. 11. Ultraviolet absorption spectra of solutions L_1, L_2, L_4 and L_5.

such as methanol, were quantitatively present in solution L_4. 75.3 per cent of the acid equivalents in solution L_1 remained in solution L_4. The rest, mainly sulfur dioxide, had escaped during the treatment of the spent liquor. The absorption of light of wave length 280 mμ by solution L_4 amounted to 94.9 per cent of the absorption of the original spent liquor and the absorption of light of wave length 380 mμ by the former after reaction with o-aminodiphenyl was 97.3 per cent of the

absorption by the latter. The compounds responsible for the losses, 5.1 and 2.7 per cent, were only partly present in solutions L_2 and L_5.

APPARATUS

Five kilograms of the strongly acid cation exchange resin Dowex-50, X-2, H^+, 100/200 mesh, were backwashed with water to remove fine-

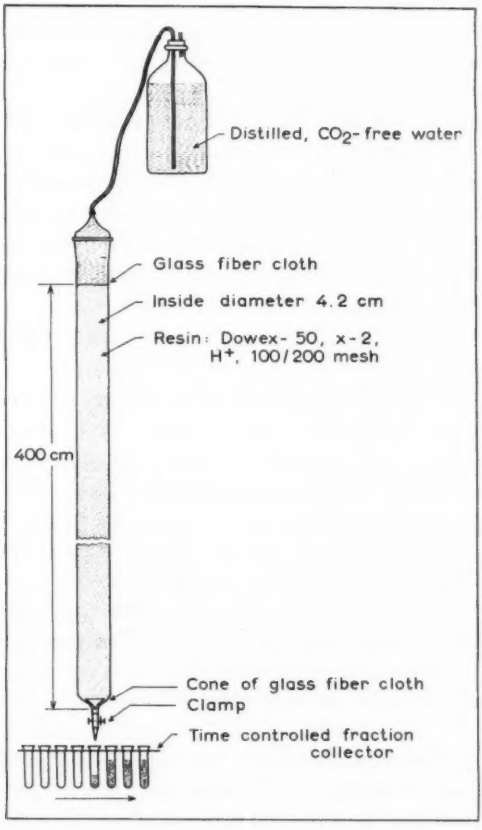


Fig. 12. Ion exclusion apparatus.

grained particles and impurities. Water-soluble impurities were removed by treating the resin alternately with 2 N sodium chloride and 2.5 N hydrochloric acid until the absorbance of the hydrochloric acid effluent in the wave length range 220—350 mμ was less than 0.03.

The ion exchange resin, which was in the acid form after this treatment, was washed free of chloride ions with deaerated CO_2-free distilled water and then transferred to a glass tube of the form shown in Fig 12.

The resin bed, which was 400 cm high and 4.2 cm in diameter, rested on a glass fiber cone in the glass tube and was covered by a glass fiber layer to prevent the added solution from splashing when it was pipetted on the column. A glass capillary tube was attached to the lower end of the former tube by a piece of plastic tubing the opening of which could be altered with a clamp to adjust the rate of flow through the column.

The apparatus included also a 10-liter glass vessel containing previously boiled distilled water and a small amount of mercury (II) iodide to prevent degradation of the solutes in the effluent by moulds.

The effluent was collected in 20-ml test tubes in a time-controlled fraction collector. A few grains of mercury (II) iodide had been added to each test tube. The drop rate was kept constant so that the collected fractions were approximately equal in volume.

FRACTIONATION 1

Twenty milliliters of solution L_4 was transferred with a pipette to the top of the ion exchange resin in the ion exclusion column and allowed to flow down the column followed by water. The timing of the fraction collector was adjusted so that the collecting test tube was replaced by an empty one every twelfth minute and the drop rate so that a fraction approximately 10 ml in volume was collected in each tube. The flow rate was then 0.06 $ml \cdot min^{-1} \cdot cm^{-2}$. The volume of the effluent in each collector tube was determined by measuring the height of the liquid in the tube and filling the test tube afterwards with water from a buret to this level. The volumes of the fractions 150—550 of Fractionation 1 are plotted in Fig. 13.

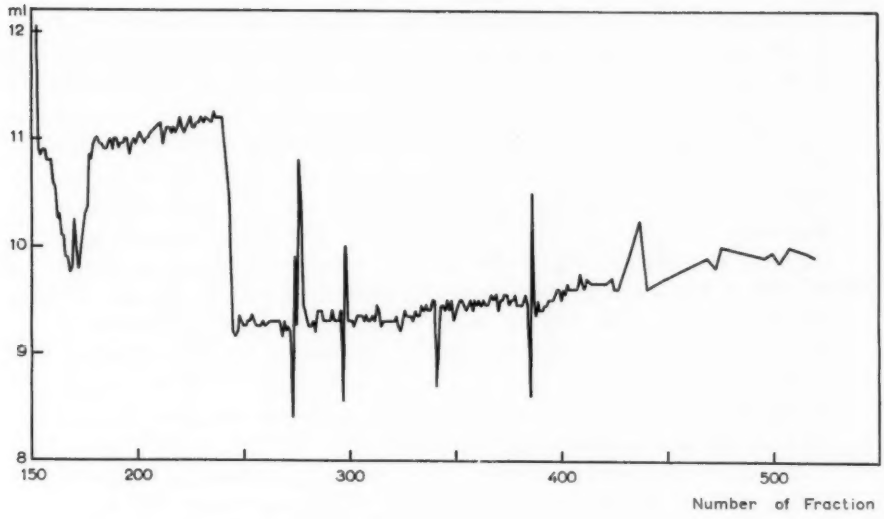


Fig. 13. Volumes of fractions of Fractionation 1.

Analysis of the Fractions

The first 155 fractions of Fractionation 1 contained water only. The following analyses were made on the subsequent fractions:

I. Measurement of absorbance at 280 mμ.

II. Determination of acidity (mequiv./ml of fraction). Two or five milliliters of each fraction was titrated with 0.0210 N sodium hydroxide employing phenolphthalein as indicator.

III. Measurement of absorbance at 380 mμ of samples treated with o-aminodiphenyl as described on p. 89.

IV. Determination of dry matter remaining after the titrated sample had been dried to constant weight at 60°C.

The results of the analyses are plotted in Fig. 14 (facing p. 94).

The aromatic compounds in spent sulfite liquor strongly absorb ultraviolet light of wave length 280 mμ. From Fig. 14 it will be seen that all these compounds, including the lignosulfonic acids, were eluted in the fractions 156—200. It is seen further that these compounds yielded two distribution curves. The major part had its absorption maximum at

fraction 170 and the minor part at fraction 185. Although the absorbance at 280 mμ was almost zero already after fraction 200, the absorbance at 380 mμ following reaction with o-aminodiphenyl revealed the existence of a large number of substances. The absorbance in the early part of the elution diagram at approximately fraction 170 was probably caused by the product of the reaction of o-aminodiphenyl with the coniferyl-aldehyde groups of the lignosulfonic acids (cf. p. 43). After this first distribution curve there followed three smaller peaks with maxima at fractions 270, 320 and 340. There then followed a very sharp maximum at fraction 390 and a shallow maximum at fraction 417. As the last two maxima were due, as will be shown later, to the monosaccharides of the spent sulfite liquor, the fractionation effected a complete separation of the lignosulfonic acids from the monosaccharides. After the monosaccharide maxima, only one peak with its maximum at approximately fraction 480 was observed.

The curve plotting the acidities of the fractions exhibited, as expected, a high peak in the early part of the chromatogram where the lignosulfonic acids were located. The later fractions consumed only small amounts of sodium hydroxide and yielded only low maxima as at approximately fraction 390 in the monosaccharide range. At the end of the chromatogram a high peak with its maximum at fraction 491 was found. It must be emphasized that any lactones present were not titrated under these conditions and hence the lactone-forming acids were only partly titrated.

The Quantitative Distribution of Matter and Reactive Groups in the Fractions

In order to facilitate the examination of the analytical data, the latter have been summed to give values for six fraction groups (I—VI) (Fig. 14). Table XII shows the quantitative distribution of dry matter, acidity, absorbance at 280 mμ and absorbance at 380 mμ after treatment with o-aminodiphenyl for the six fraction groups. The figures give the values as percentages of the values obtained for solution L_4.

As shown by the data in Table XII, the substances in solution L_4 were almost quantitatively found in the Fraction Groups I—VI and hence no significant irreversible absorption had occurred during the passage through the ion exchange resin.

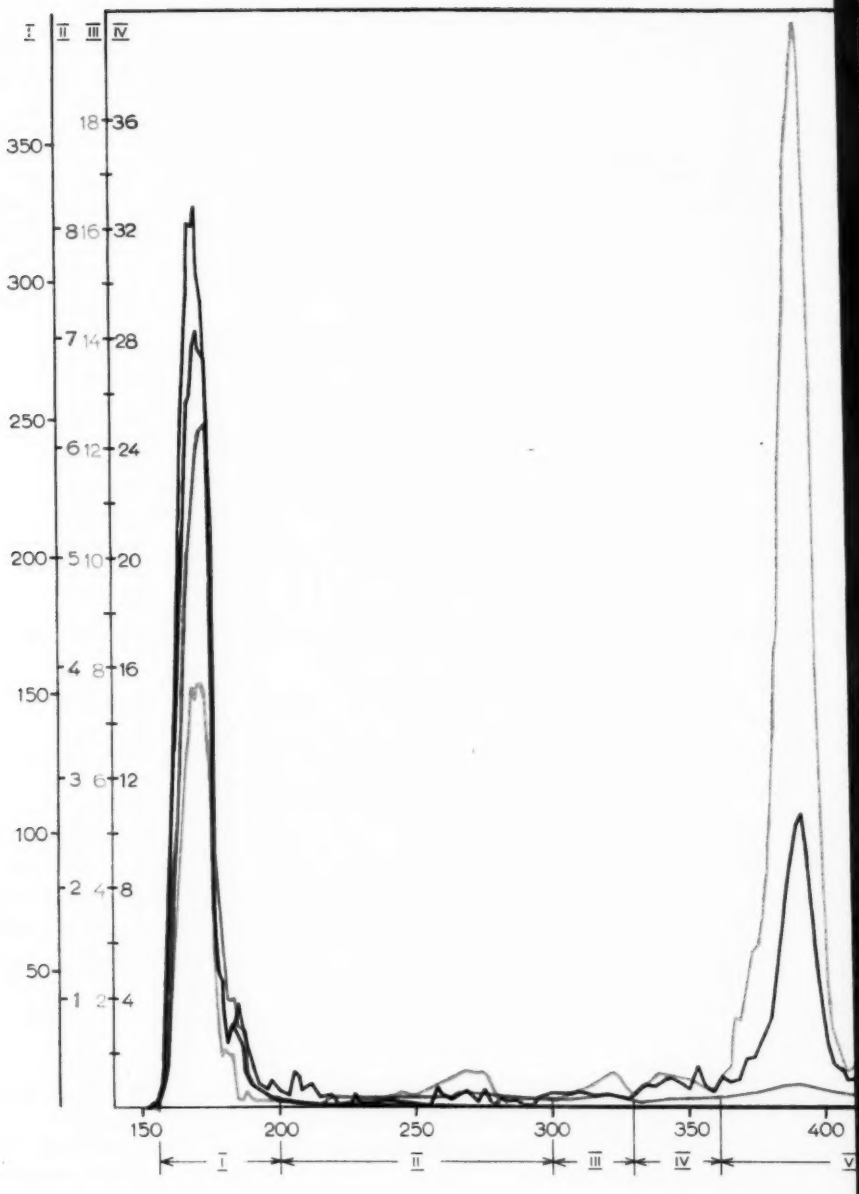
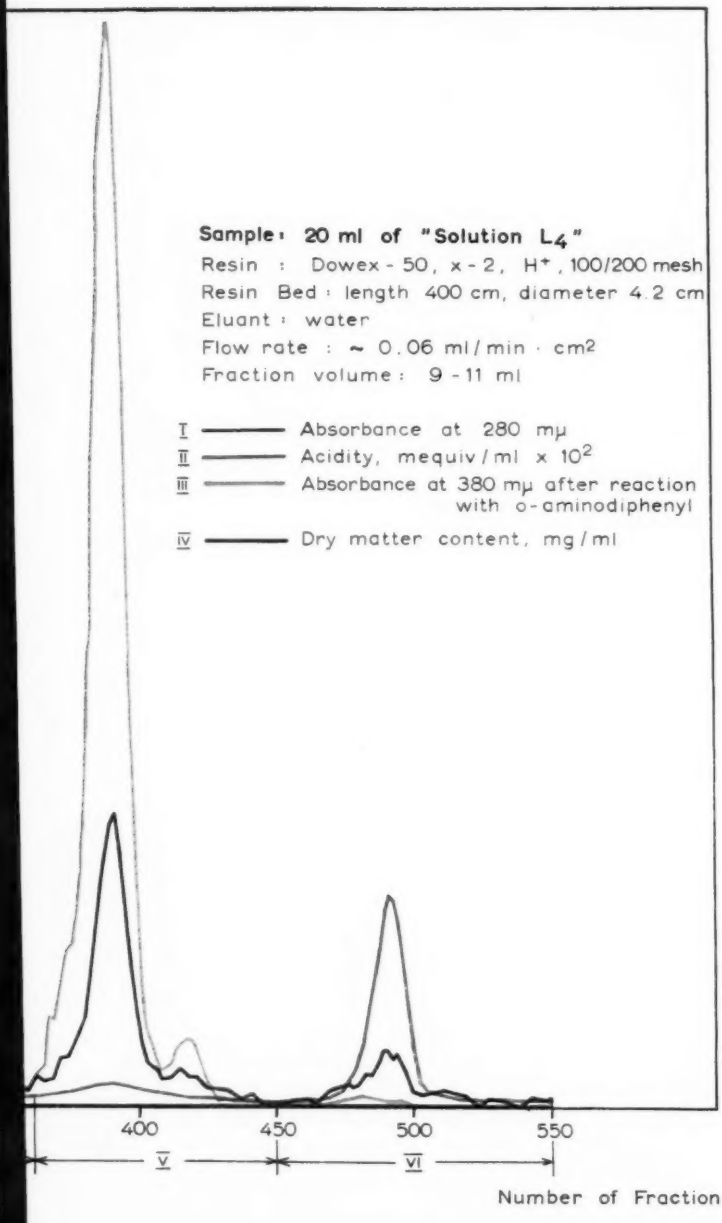


Fig. 14. Elution Diagram 1. Analytical data relatin



ical data relating to Fractionation 1.

Table XII.

Analytical Data for Fraction Groups I—VI of Fractionation 1 Expressed as Percentages of the Corresponding Values for Solution L_4

Fraction Group	Fractions nos.	Dry matter content %	Acid equivalents %	Absorbance at 280 mμ %	Absorbance at 380 mμ after reaction with o-aminodiphenyl %
I	156—200	60.0	59.0	94.2	21.6
II	201—300	2.5	5.6	1.3	4.5
III	301—329	1.0	1.4	0.1	1.9
IV	330—362	3.2	1.4	0.1	2.9
V	363—449	26.1	5.6	0.0	61.8
VI	450—550	5.7	19.0	0.0	1.3
Total		98.5	92.0	95.7	94.0

Examination of Fraction Groups I—VI

Fraction Group VI

The main substance in this fraction group was acetic acid, which was most highly concentrated in fraction 491 and adjoining fractions (Fig. 14). In Fig. 15 the part of Elution Diagram 1 relating to Fraction Group VI is drawn on a larger scale.

As seen from Fig. 15, small amounts of neutral compounds with maxima at fractions 480 and 510 were present in addition to acetic acid. The first-mentioned peak was revealed by a rose color resulting from the reaction with o-aminodiphenyl.

Fraction Group V

This fraction group contained the monosaccharides of the spent sulfite liquor. The monosaccharides were separated further by chromatographic analysis of aliquots of the fractions on Whatman No. 1 filter

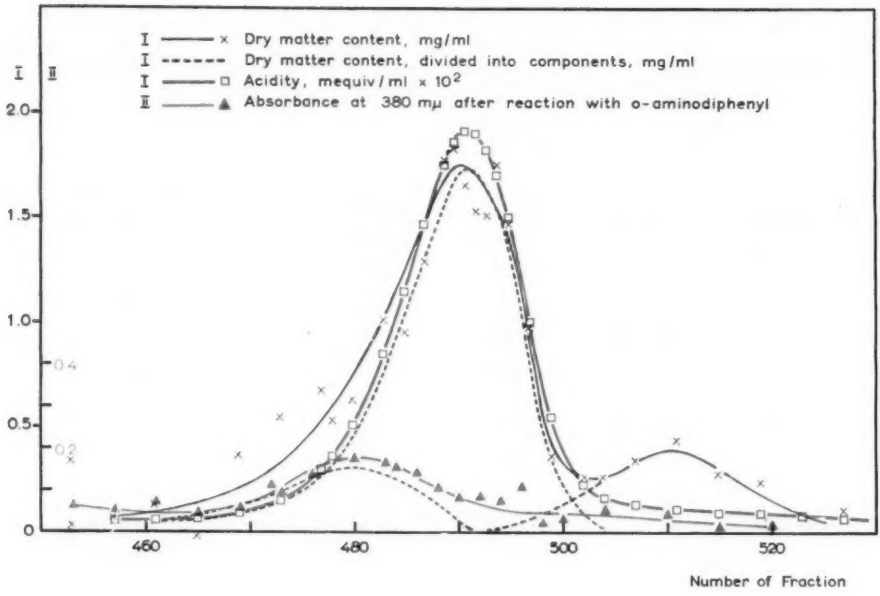


Fig. 15. Analytical data for Fraction Group VI.

paper with ethyl acetate-pyridine-water (8:2:1) during 30 hours. The isolated monosaccharides were then determined quantitatively according to Piper and Bernardin.[229] The distribution curves thus obtained showed that the monosaccharides were partly separated in Fractionation 1 (Fig. 16).

To determine the amount of compounds other than monosaccharides in Fraction Group V, the sum of the monosaccharides was subtracted from the total dry matter. The differences, plotted in Fig. 16, indicate that Fraction Group V contained appreciable amounts of other compounds. As seen in Fig. 14, the Fraction Group V contained also substances that consumed a small amount of sodium hydroxide when the fractions were titrated to the phenolphthalein end point. As it was suspected that these compounds were aldonic acids that exist largely in lactone form in aqueous solution, 5 ml of 0.01 N sodium hydroxide solution was added to 2 ml of each fraction in a nitrogen atmosphere to saponify the lactone rings and the sodium hydroxide in

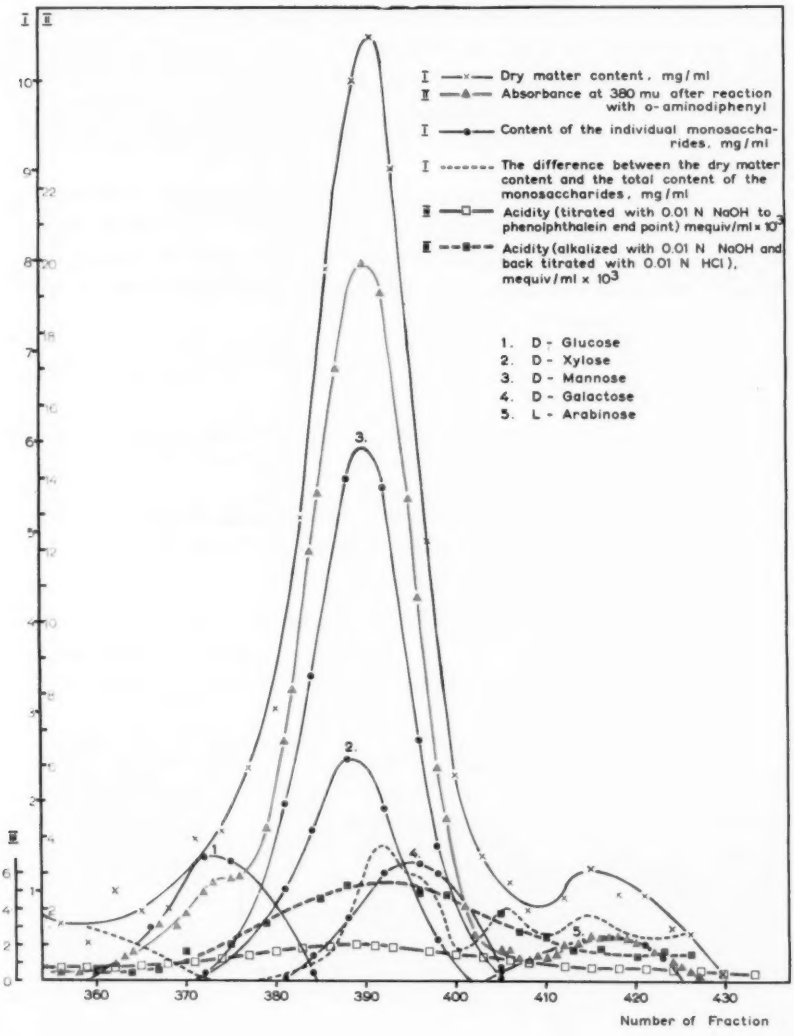


Fig. 16. Analytical data for Fraction Group V.

excess was titrated with 0.01 N hydrochloric acid to pH 8. The results, which are plotted in Fig. 16, support the view that the unknown substances in Fraction Group V were aldonic acids that were partly present in lactone form and show further that these substances were roughly distributed according to the dry weight difference curve mentioned above.

The contents, as determined by paper chromatography, of the five monosaccharides found in Fraction Group V are given in Table XIII. The content of uninvestigated compounds was obtained by taking the difference between the total dry matter content and the sum of the monosaccharide contents.

Table XIII

Distribution of Dry matter in Fraction Group V of Fractionation 1

Fractions	Compound	Content as percentage of total dry matter in solution L_4
360—384	D-Glucose	2.6
370—402	D-Xylose	4.6
370—405	D-Mannose	11.0
381—405	D-Galactose	2.6
404—425	L-Arabinose	0.9
363—449	Not investigated	4.4

Fraction Groups III and IV

As shown by the curves in Fig. 14, the Fraction Groups III and IV contained small amounts of compounds which reacted with o-aminodiphenyl. A part of Fig. 14 is drawn on a larger scale, which reveals more details, in Fig. 17.

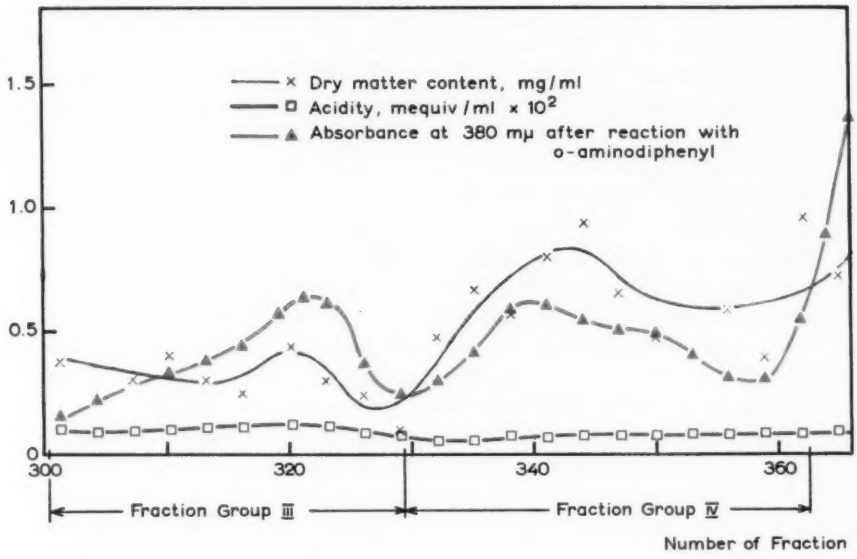


Fig. 17. Analytical data for Fraction Groups III and IV.

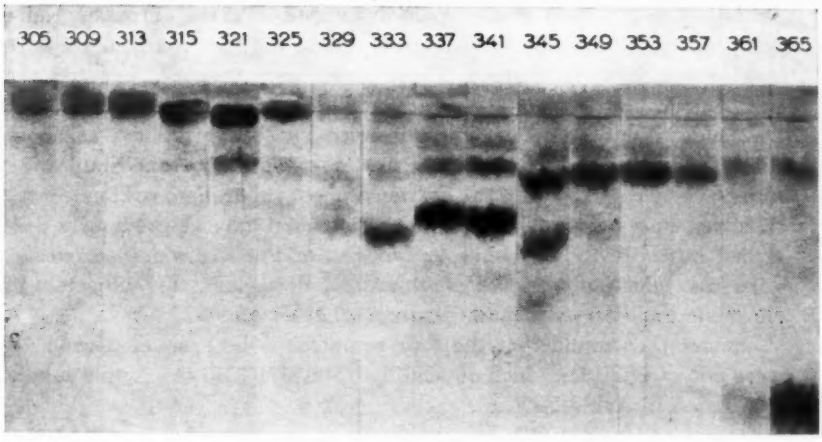


Fig. 18. Paper chromatograms of various fractions of Fraction Groups III and IV.

As the fraction groups could be assumed to contain polysaccharides, the following investigations were carried out. One-milliliter aliquots of 16 of the fractions 305—365 were separately subjected to paper chromatography on Whatman No. 1 paper employing ethyl acetate-pyridine-water (8:2:1) for 72 hours and then sprayed with an aniline oxalate solution and heated five minutes at 115°C. A photograph of the paper chromatograms taken in ultraviolet light is reproduced in Fig. 18.

As seen from Fig. 18, the Fraction Group IV, which is represented by fractions 333—365, was found by paper chromatography to contain four components* of which the second and third from the starting line were present in largest amount. It is seen further that these two components were most strongly concentrated around fractions 349 and 340, respectively. This can also be seen from Fig. 17. The spots in the lower right-hand corner in Fig. 18 are due to glucose (cf. Fig. 16).

Fraction Group III, which is represented by fractions 305—329 in Fig. 18, contained one component that did not move from the starting line and a second component that migrated about 4 cm. Both these components appeared to be concentrated about fraction 321 (compare Fig. 17). The former of the components appeared to be present in higher concentration.

In order to study the compositions of the four components in Fraction Group IV, three 1-ml samples of fraction 342 were simultaneously chromatographed on the same paper by the procedure described above. The two outermost chromatograms were then made visible with aniline oxalate. On the basis of the spots developed in these chromatograms, the parts of the central chromatogram where the four components were located could be determined. These areas were then cut out and the components eluted from them with distilled water. Hydrochloric acid was added to the aqueous extracts until its concentration was about 0.1 N, and the extracts were then heated at 118°C for one hour in an autoclave and evaporated to dryness. The evaporation residues were dissolved in water and subjected to chromatography as previously described but only for 22 hours. A mixture of the five monosaccharides galactose, glucose, mannose, arabinose and xylose was subjected to chromatography on the same paper (Fig. 19).

It was thus found that the four components in Fraction Group IV were polysaccharides which on acid hydrolysis yielded the simple sugars mentioned in Table XIV.

* The term »component» as used here does not necessarily refer to a single compound, but may refer to a mixture of compounds that behaved similarly in the separation process.

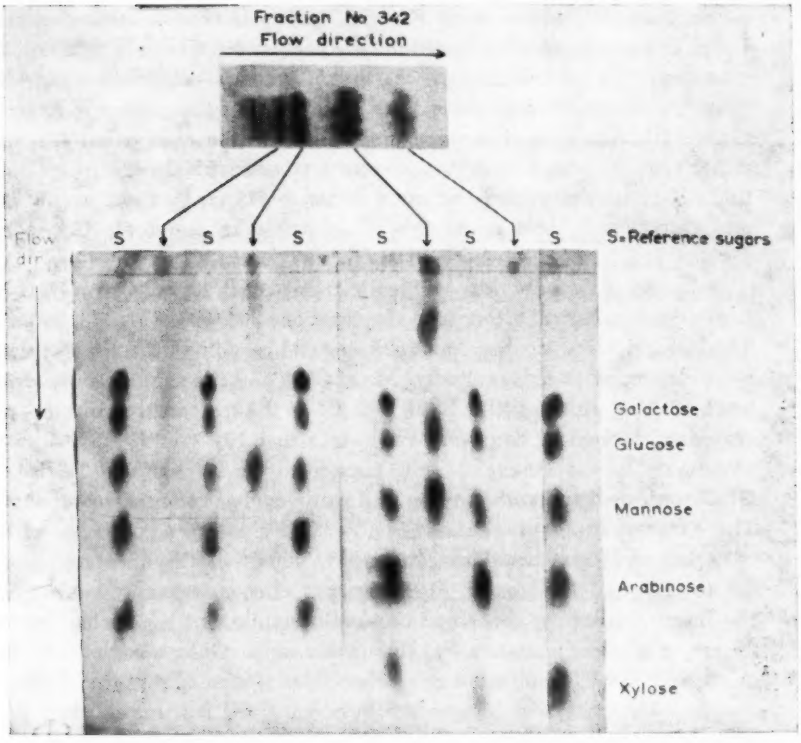


Fig. 19. Paper chromatograms of the four components of fraction 342 of Fraction Group IV after hydrolysis.

Table XIV.

Monosaccharides Obtained by Hydrolyzing the Four Components of Fraction 342

Spot 1	Spot 2	Spot 3	Spot 4
Glucose Mannose Galactose	Mannose Glucose	Glucose Mannose	Xylose

Fraction 321 representing Fraction Group III was investigated similarly. The previously mentioned mobile component contained glucose and mannose. The part of fraction 321 that was retained on the starting line probably represented, however, the major part of the matter in Fraction Group III. Attempts that were made to elute the component from the paper chromatogram with water were unsuccessful. On the other hand, this component migrated the same distance (15 cm) as glucuronic acid and galacturonic acid when it was subjected to paper electrophoresis (1000 volts, 1 hour, 0.1 N NaOH).

In order to study this acid component of Fraction Group III, the titrated and weighed aliquots of the fractions 304, 307, 310, 313, 316, 320, 323, 326 and 329 were dissolved in water and combined. Lactones present were cleaved with sodium hydroxide at pH 9 and the solution evaporated to about 1 ml on the water bath. 0.05 ml of the evaporated solution was chromatographed 40 hours on Whatman paper No. 1 with ethyl acetate-pyridine-water-acetic acid (5:5:3:1) according to Fischer and Dörfel.[230] Glucuronic acid and galacturonic acid were used as reference compounds. The chromatograms were made visible with a solution composed of 2 g of aniline and 2 g of trichloroacetic acid in 100 ml of ethyl acetate according to Gee and McCready.[231] The paper chromatogram revealed that the fraction mixture contained one main component which had moved 28 cm, the same distance as glucuronic acid. Galacturonic acid had migrated 25 cm. The fraction mixture yielded a faint spot at this distance. Beside these spots, which were brown in color, the fraction mixture gave three spots at distances 20, 37 and 46 cm from the starting line. Of these the last two were brown in color. The first spot was clearly visible only in ultraviolet light.

The acid properties which were revealed by orientative paper electrophoretic studies are not clearly revealed by Fig. 17 which shows only an insignificant increase in acidity at fraction 320. As the paper chromatogram showed, it is possible that the main component of Fraction Group III consisted of glucuronic acid which forms a lactone in water and is hence not titrated with sodium hydroxide to the phenolphthalein end point. Galacturonic acid, on the other hand, does not form a lactone in water.[230]

Is was mentioned on p. 20 that glucuronic acid, 4-O-methyl-glucuronic acid and galacturonic acid are the uronic acids found in spruce wood. It was therefore of interest to determine whether 4-O-methyl-glucuronic acid was also a component of Fraction Group III. The methoxyl content of the dry matter (sodium salts) of the combined fractions 317—318 was therefore determined. The found methoxyl content was, however,

only about 0.4 per cent (0.54; 0.33). If these methoxyl groups were derived from the mentioned uronic acid, this must have been only a minor component of Fraction Group III.

Fraction Group II

As shown by Fig. 14, the fractions 240—280 displayed a relatively high reactivity towards o-aminodiphenyl. The acid properties are revealed by the results of the acidimetric titrations. The fractions did not, however, absorb light of wave length 280 mμ. Methoxyl content determinations on the dry matter of the combined fractions 261—262 and 273—280 gave the results shown in Table XV.

Table XV

Methoxyl Contents of the Dry Matter (Na salts) of Some Fractions of Fraction Group II of Fractionation 1

Sample	Fractions	Methoxyl content, %
1	261—262	4.23; 4.18
2	273—280	3.82; 3.31

The data show that the fractions 240—280 contained at least a part of the nonaromatic methoxyl-containing matter of solution L_4. As mentioned on p. 20, the methoxyl groups not bound to aromatic compounds are mainly present as 4-O-methyl-D-glucuronic acid. The methoxyl content of the sodium salt of this acid is, however, 12.66 %. On the assumption that the methoxyl groups present are those in the above acid, the observed low methoxyl content implies that the fractions 240—280 contained also methoxyl-free compounds.

The existence of such methoxyl-free compounds was studied by heating 0.5 ml of fraction 267 with an equal volume of 2 N sulfuric acid in an autoclave at 120 C° for one hour. The solution was then neutralized with barium carbonate and filtered. The filtrate was passed through a column of the cation exchange resin Dowex-50, X-8, H^+, 50/100 mesh, which was 8 cm high and 0.7 cm in diameter. The effluent was evaporated to near dryness on a water bath and then chromatographed on What-

man No. 1 paper during 24 hours with a mixture of ethyl acetate-pyridine-water (8:2:1). On the same paper were chromatographed 0.5 ml of the unhydrolyzed fraction and a volume of reference solution containing 0.1-mg quantities of the monosaccharides galactose, glucose, mannose, arabinose and xylose. The reducing components were rendered visible with aniline oxalate (Fig. 20).

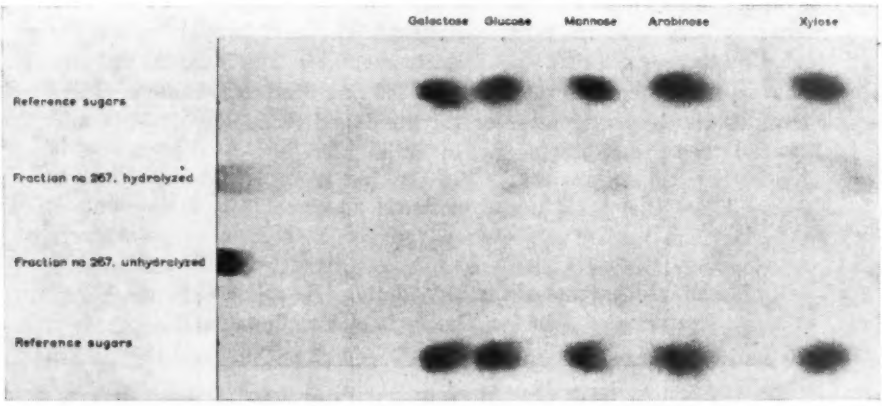


Fig. 20. Paper chromatogram of fraction 267 of Fraction Group 11 before and after acid hydrolysis.

From the chromatogram it is seen that the acid hydrolysis liberated xylose and small amounts of glucose and mannose from fraction 267. The compounds of the unhydrolyzed fraction that reacted with aniline oxalate had not moved from the starting line.

If it is assumed that the fraction 267 did not contain non-reducing sugars, the immobility of the compounds in the unhydrolyzed fraction that reacted with aniline oxalate was due either to their being high-molecular neutral sugars or to the fact that the monosaccharides liberated by hydrolysis were bound to acid compounds such as the methyl-substituted uronic acid mentioned above.

To determine whether the compounds in the unhydrolyzed fraction that reacted with aniline oxalate were bound to acids, fraction 265 was subjected to paper electrophoresis as follows. Half a milliliter of the fraction was made alkaline with 0.01 N sodium hydroxide to open up any lactone rings present and the solution then transferred to a strip of Whatman No. 3 MM paper 61 cm long. To the same strip was added

a solution containing 0.1 mg of glucose and on each side of these a solution containing about 0.2 mg of glucuronic acid. The glucuronic acid solution had previously been made alkaline to hydrolyze any glucuronolactone present. The electrophoresis was carried out employing an acetate buffer of pH 4 in an apparatus of the Kunkel-Tiselius type. The development time was one hour and the applied voltage 2000 V. The reducing substances were made visible with aniline oxalate (Fig. 21).

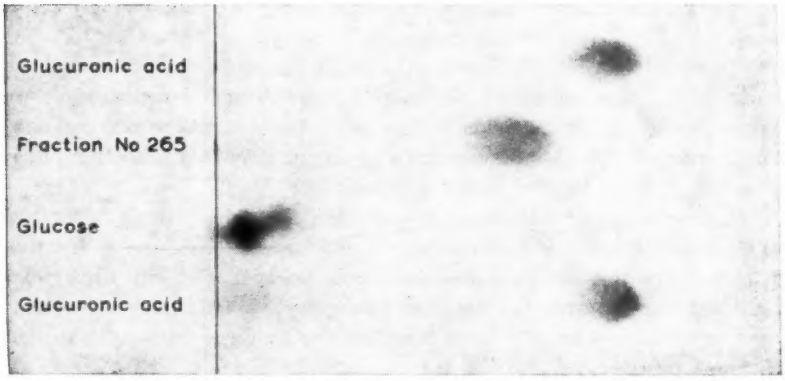


Fig. 21. Paper electrophoresis of fraction 265 of Fraction Group II.

It will be seen from the figure that practically all of the compounds in the unhydrolyzed fraction that reacted with aniline oxalate migrated during the electrophoresis. It is therefore probable that the xylose present was bound to acids. The major part of the reducing substances in fraction 265 had migrated 12 centimeters. These components reacted with aniline oxalate to give a spot that was orange-colored in ultraviolet light and resembled that obtained with O-(4-O-methyl-α-D-glucosyluronic acid)-(1→2)-D-xylose **(12)** (p. 21). A second weak spot was found 15.5 cm from the starting line at the same distance as the spot due to glucuronic acid. Both these spots were colored brown in ultraviolet light. The reference compound glucose had migrated 1 cm by electro-osmosis during the same time. Both the fraction 265 and glucuronic acid gave very weak spots at a distance of 1 cm from the starting line that were evidently due to lactones.

The ratio of the distances the main component of fraction 265 and glucuronic acid had migrated was, after a correction was applied for

electro-osmosis, 0.76. The ratio found by Theander [232] for O-(4-O-methyl-α-D-glucosyluronic acid)-(1→2)-D-xylose and glucuronic acid was 0.74.

These experiments thus indicated that the Fraction Group II probably contained the mentioned aldobiuronic acid.

Fraction Group I

The part of Elution Diagram 1 arising from the fractions 152—200 is shown in Fig. 22 on a scale that reveals more details than Fig. 14.

As seen from Fig. 22, Fraction Group I contained the compounds in solution L_4 that absorbed ultraviolet light. These compounds were primarily lignosulfonic acids which gave an absorption maximum at about fraction 170. Smaller amounts of compounds that absorbed ultraviolet light were located in the fractions 180—190.

It may be noted that the curve plotting the acidity extends further to the right than the absorbance curve and has its maximum at fraction 173. Had Fraction Group I contained only acids that absorb ultraviolet light, the acidity curve and the absorbance curve should have had coincident maxima and should have been similar in form with ordinates in constant ratio.

From the figure it is seen that the ratio of absorbance to acidity is constant up to the fraction 170 and then begins to decrease. This implies that the fractions 156—170 contained uniform matter (lignosulfonic acids), while the fractions 171—180 contained in addition acids that do not absorb light of wave length 280 mμ.

That the fractions 156—170 contained only lignosulfonic acids was confirmed by methoxyl determinations which revealed that the methoxyl contents of the dry matter (sodium salts) in these fractions (Table XVI) were fairly constant.

Table XVI

Methoxyl Contents of the Dry Matter (Sodium Salts) of Some Fractions of Fraction Group I of Fractionation 1

Fraction	Methoxyl content, %
163	10.94; 10.86
166	10.48; 10.68
172	10.11; 10.35

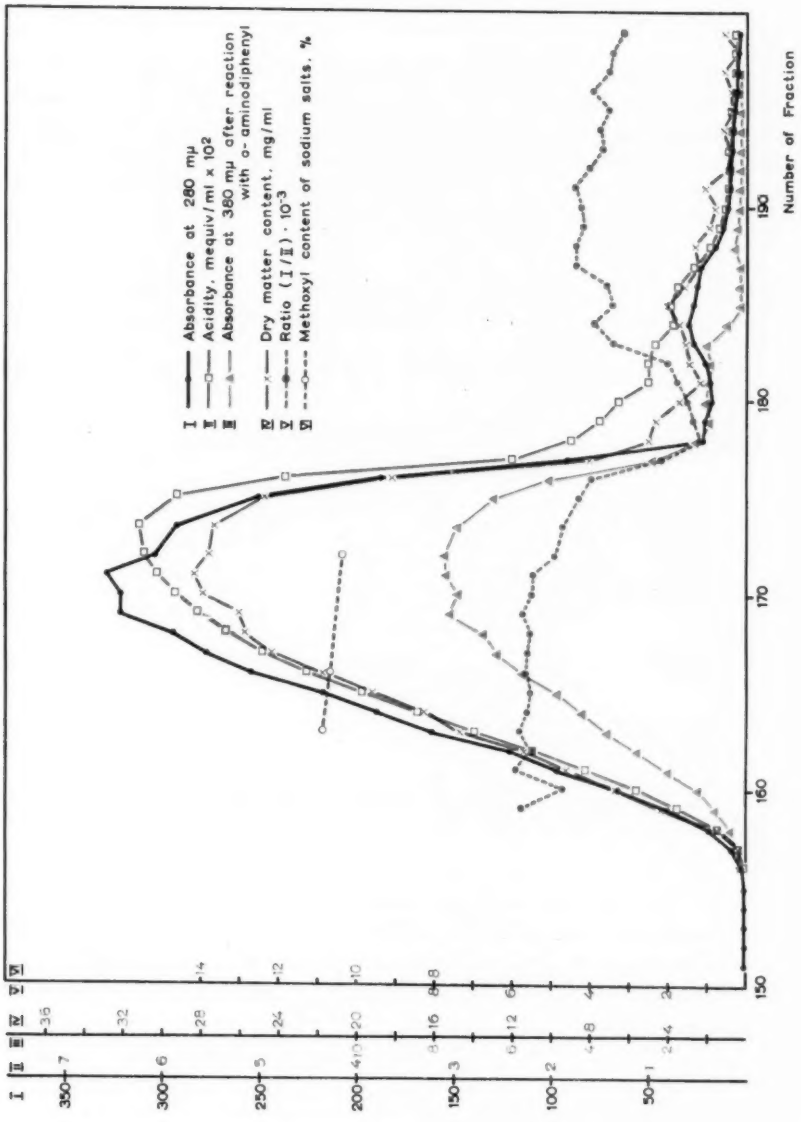


Fig. 22. Analytical data for Fraction Group I.

FRACTIONATION 2

In order to achieve a better separation of the lignosulfonic acids and the acids that did not absorb ultraviolet light (p. 106), a new sample was fractionated at a lower flow rate. Only the fractions that approximately corresponded to Fraction Group I of Fractionation 1 were collected. The volumes of the fractions were determined as described on p. 92 and are plotted in Fig. 23.

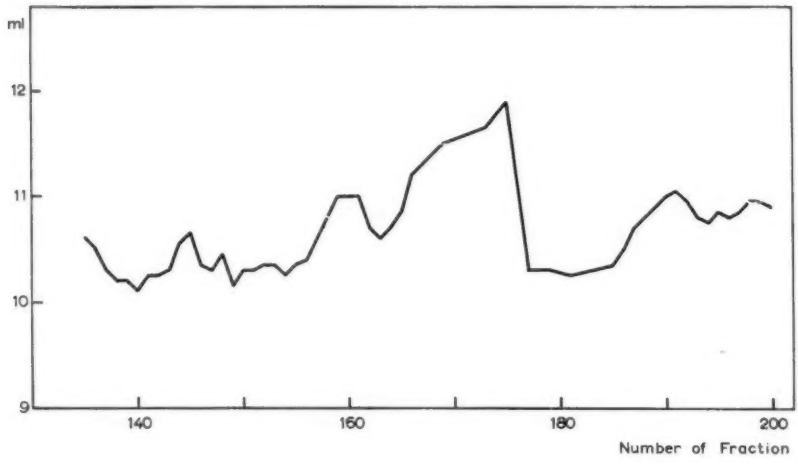


Fig. 23. Volumes of fractions of Fractionation 2.

Each fraction was collected over a period of 48 minutes, which corresponds to a mean flow rate of 0.015 ml · min^{-1} · cm^{-2}, a rate that was only a fourth of the flow rate when Fractionation 1 was carried out. In all other respects the conditions were the same as when Fractionation 1 was performed.

Analysis of the Fractions

The first 134 fractions contained water only. The following analyses were made on the subsequent fractions:

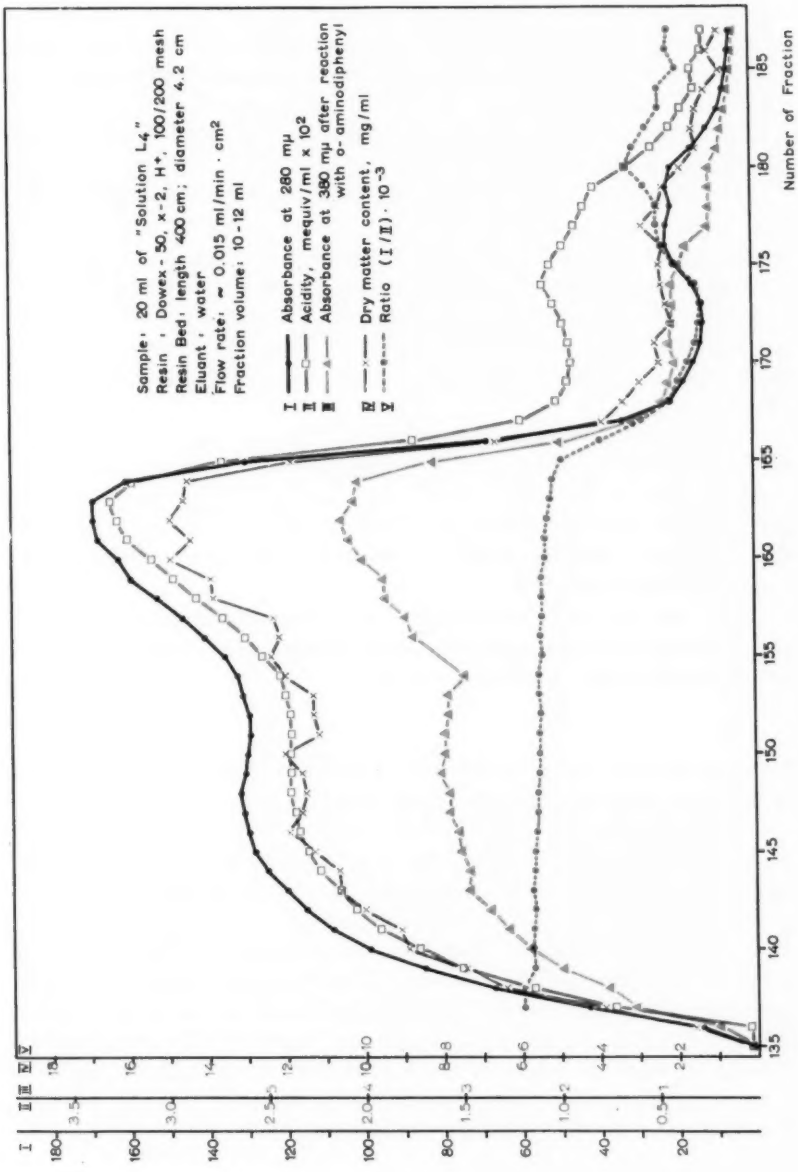


Fig. 24. **Elution Diagram 2. Analytical data relating to Fractionation 2.**

I. Measurement of absorbance at 280 mμ.
II. Determination of acidity. Three milliliters of each fraction were titrated with 0.0100 N sodium hydroxide employing bromthymol blue as indicator.
III. Measurement of the absorbance at 380 mμ after reaction with o-aminodiphenyl (p. 89).
IV. Determination of dry matter content after evaporating and drying the titrated sample at 55°C.

The results of these analyses are plotted in Fig. 24.

The greatest difference between the elution diagrams of Fraction Group I of Fractionation 1 and Fractionation 2 is that in the latter the lignosulfonic acids were partly separated into two groups, one with its maximum at fraction 148 and another with its maximum at fraction 163.

The ratio of the absorbance at 280 mμ and the acid content is also constant in Elution Diagram 2, but extends over a greater number of fractions than in the case of Fraction Group I of Fractionation 1. The fact that the ratio is constant over two distributions strongly supports the assumption that the fractions 135—163 contained compounds with closely similar properties.

As in the case of Fractionation 1, small amounts of compounds that absorbed ultraviolet light with absorbance maxima at fractions 176 and 179 in Fractionation 2 were also detected.

Resolution of the Property Distribution Curves

Property Distribution Curves of Components A and B and the Absorbance (280 mμ) Distribution Curves of Components C and D

It may be tentatively assumed that the absorbance curve I in Fig. 24 is composed of four distribution curves with maxima at the fractions 148, 163, 176 and 179. The first two of these distribution curves, which will be referred to as relating to components A and B, may be taken to be similar in form. The data for fractions 135—148 plot the left slope for component A with its maximum at fraction 148, and in accordance with this the data for fractions 150—163 plot the left slope for component B with its maximum at fraction 163.

Ratios of the analytical data for corresponding fractions of components A and B are plotted in Fig. 25.

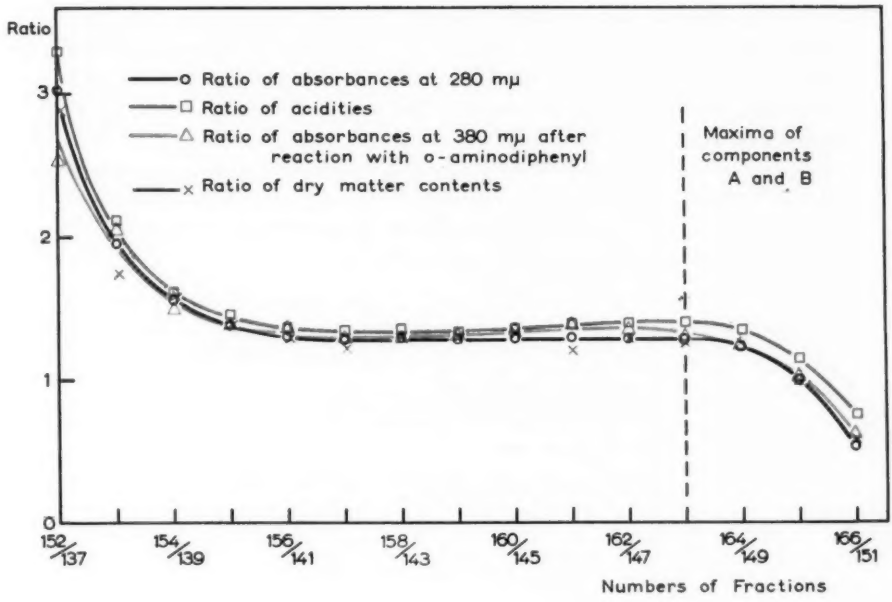


Fig. 25. Ratios of analytical data for corresponding fractions of components A and B.

It is seen from Fig. 25 that the distribution curves of components A and B in Fig. 24 are similar in form and that the analytical values for the fractions containing component B are about 1.28 times the values for the fractions containing component A. It is observed further that the fractions 157—163 contained only the ultraviolet-light-absorbing compounds that belonged to component B and that fractions 142—148 contained only ultraviolet-light-absorbing compounds that belonged to component A. Thus, by multiplying the measured values for fractions 135—148 (left slope of A) by 1.28, the left slope of the absorbance curve of B was obtained and subtraction of the resulting values from the measured absorbances of fractions 150—163 gave the right slope of A. In this way the complete absorbance distribution curve for A and the left half of the curve for B were obtained. The distribution curves for component A and the left halves of the distribution curves for component B in respect of acidity, absorbance at 380 mμ after reaction with o-aminodiphenyl and dry matter content were obtained in a similar manner. The fractions

following fraction 163 that gave the right slope of the curve for component B contained also the ultraviolet-absorbing compounds whose distribution maxima were located at fractions 175 and 179 and also the acids that did not absorb ultraviolet light and which were found to be present in Figs. 22 and 24.

The right slope of the absorbance (280 mμ) curve for component B and the complete curves for component C with its absorption maximum at fraction 175 and the component D with its maximum at fraction 179 were obtained graphically as shown in Fig. 26.

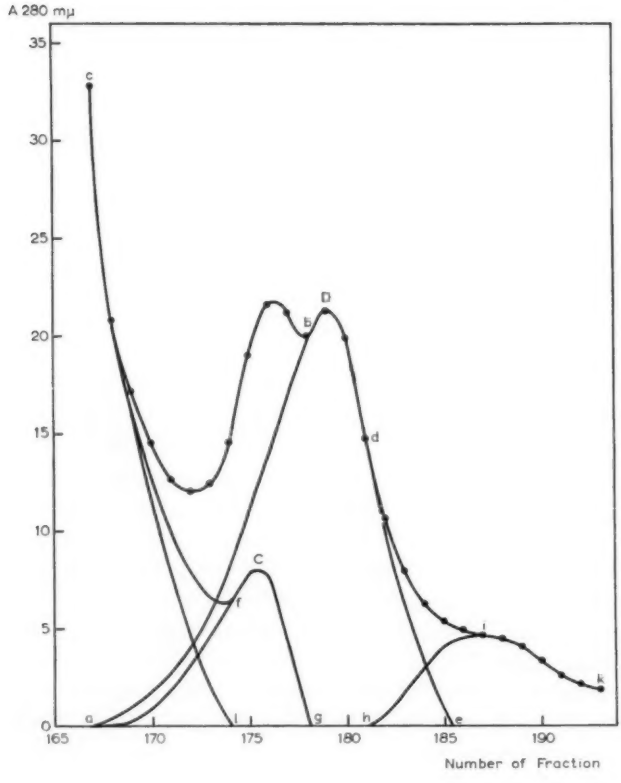


Fig. 26. The absorbances at 280 mμ of components B (fractions 167—174), C and D.

The curve *cbdik* in the figure plots the measured total absorbances of these fractions. Assuming that the left slope of the distribution curve for component D represents as many fractions as the left slopes of A and B, the curve *ab* was drawn. Curve *de* was then drawn. The resulting curve for the component D was then subtracted from the analytically determined curve *cbdik:*

$$cbdik - abde = cfghik$$

The distribution curve *(afg)* for component C was then drawn. Subtraction of the distribution curve for C gave finally the curve *cl* for component B in the fraction range 167—174.

The ratios of the absorbances at 280 mμ to the dry matter contents, the acid contents, and the absorbances at 380 mμ after reaction with o-aminodiphenyl were practically constant for the fractions containing components A and B and had the following values:

$$\frac{A_{280\ m\mu}}{\text{dry matter content, (mg/ml)}} = 11.4$$

$$\frac{A_{280\ m\mu}}{\text{acidity, (mequiv/ml)}} = 5.37 \cdot 10^{3}$$

$$\frac{A_{380\ m\mu\ \text{(o-aminodiphenyl)}}}{A_{280\ m\mu}} = 2.46 \cdot 10^{-2}$$

As the right slope of the absorbance curve for the component B was now known, the curves plotting dry matter content, acid content and absorbance at 380 mμ after reaction with o-aminodiphenyl were computed with the aid of the above ratios.

Distribution of Mass among Components C, D, E and F

The ratios of the absorbance at 280 mμ to the dry matter content in fractions 135—192 are plotted in Fig. 27.

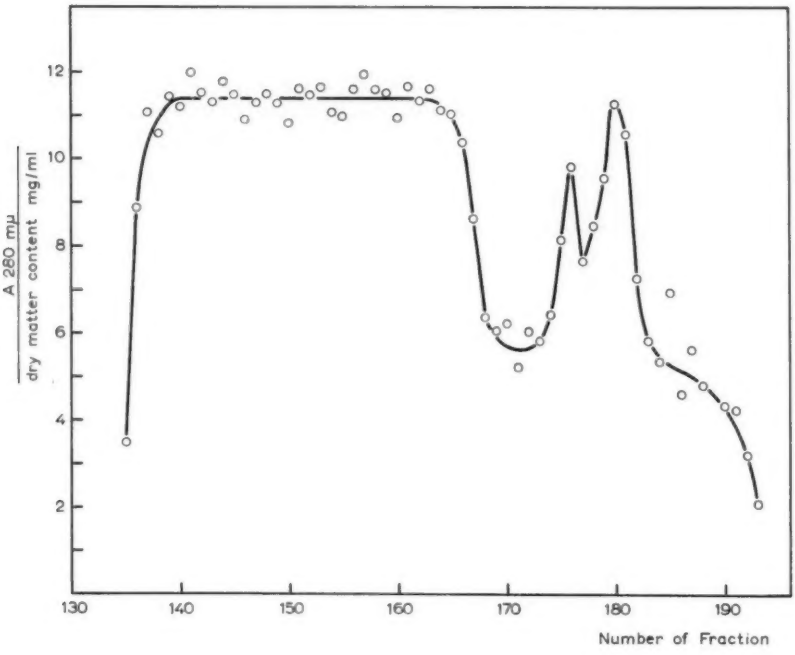


Fig. 27. Ratio of absorbance at 280 mμ to dry matter content in fractions 135—192 of Fractionation 2.

This curve shows that the ratio was constant (11.4) for the components A and B but then decreased in value owing to the effect of substances that did not absorb ultraviolet light. Following an absorption minimum at fraction 171, there is one maximum at fraction 176 due to the component C and another at fraction 180 due to the component D.

The ratio of the absorbance to dry matter content in fraction 180 was approximately the same as the ratio in the fractions containing components A and B. It was therefore assumed that fraction 180 contained only compounds belonging to component D and that the absorptivity (a_{280}) of the component D was 11.4 ($l \cdot g^{-1} \cdot cm^{-1}$). The absorptivity ($a_{280}$) of component C was assumed to be 20 ($l \cdot g^{-1} \cdot cm^{-1}$). This value was taken as an approximate value for lignans such as α-conidendrin and matairesinol.[233]

When the dry matter contents of the fractions containing components A and B were subtracted from the experimental dry matter contents, the curve *abcde* in Fig. 28 was obtained.

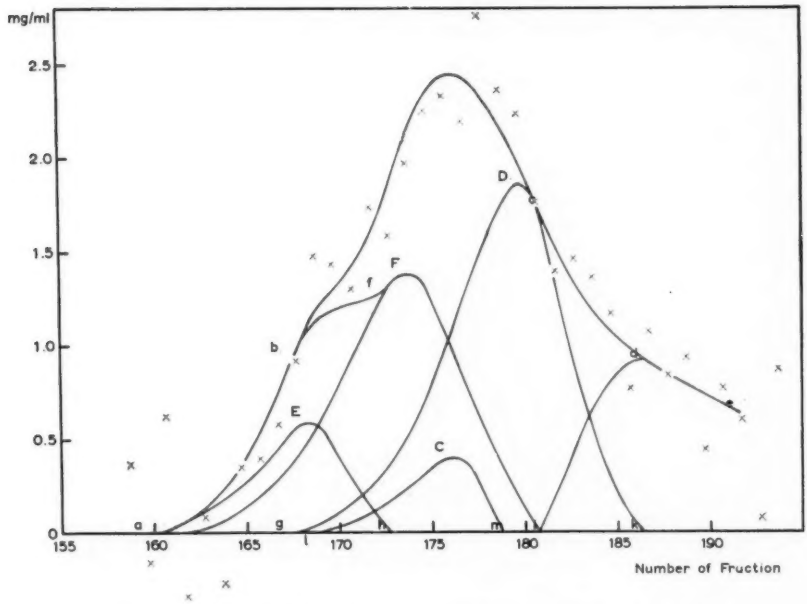


Fig. 28. Dry matter distribution among components C, D, E and F.

On the basis of the absorbance curves (280 mμ) for the components C and D and the absorptivities 11.4 and 20 ($l \cdot g^{-1} \cdot cm^{-1}$), the dry matter curves *lm* and *gck* for the components C and D were derived. By subtracting these curves from curve *abcde* and drawing curve *af*, the dry matter distribution of component F (*afi*) was obtained.

$$abcde - (lm + gck) = abfide$$

Subtraction of the dry matter curve for component F gave the dry matter distribution curve *ah* of component E:

$$abfi - afi = ah$$

Distribution of Acidity among Components D, E and F

By subtracting the acidity distribution curves for components A and B from the experimentally determined total acidity distribution curve the curve *abcde* in Fig. 29 was obtained.

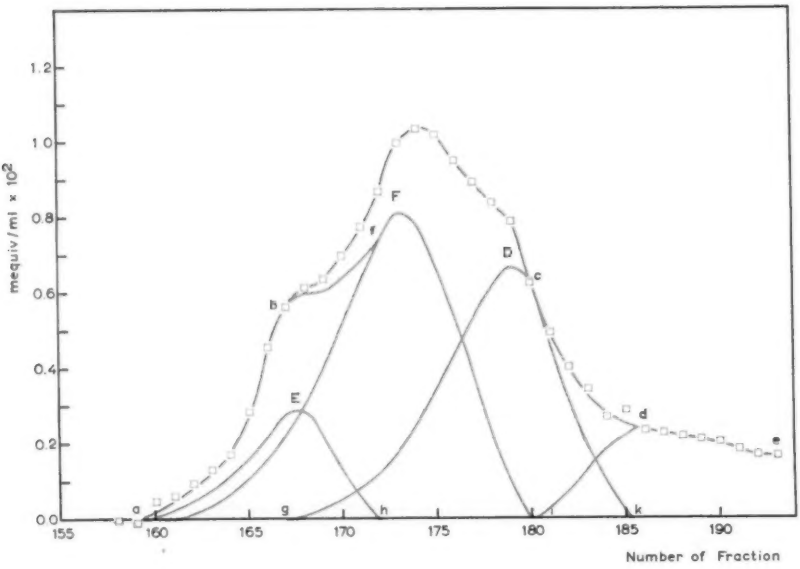


Fig. 29. Acidity distribution among components D, E and F.

The ratio of absorbance (A_{280}) to acidity (mequiv./ml) in fraction 180 was $3.17 \cdot 10^3$. On the basis of this value and the absorbance curve for D (Fig. 26), the acidity distribution curve *gck* for component D was obtained. The acidity curve for component F was obtained by subtracting the curve *gck* for component D from the total curve *abcde* and then drawing *af*.

$$abcde - gck = abfide$$

The acidity distribution curve *ah* for component E was finally obtained by subtracting the distribution curve for component F:

$$abfi - afi = ah$$

Distribution of the Absorbance at 380 mμ after Reaction with o-Aminodiphenyl among Components D, E and F

When the curves plotting the absorbance at 380 mμ after reaction with o-aminodiphenyl for the components A and B were subtracted from the experimental curve, the curve *abcde* in Fig. 30 was obtained.

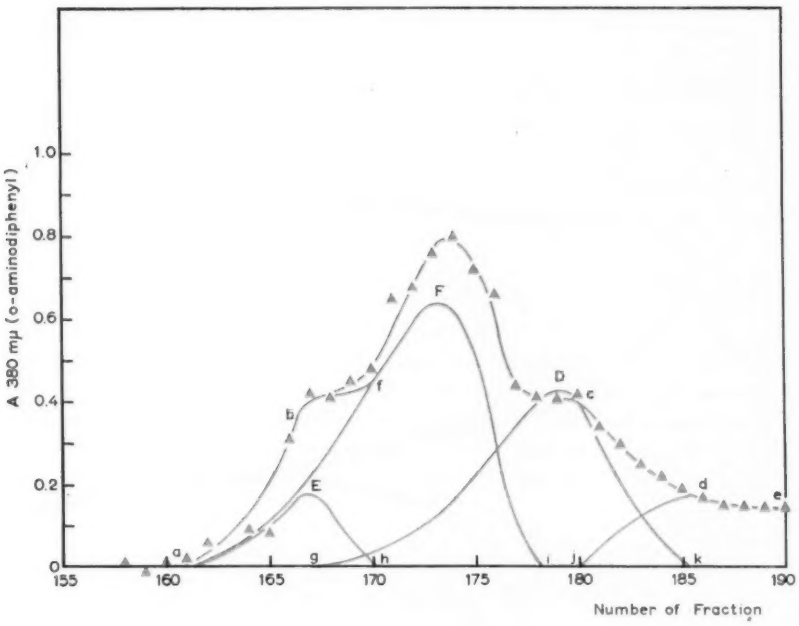


Fig. 30. Distribution of the absorbance at 380 mμ after reaction with o-aminodiphenyl among components D, E and F.

The curve *gck* plotting the absorbance at 380 mμ after reaction with o-aminodiphenyl for component D was obtained from the absorbance (280 mμ) curve for D (Fig. 26) by multiplying the ordinates by the ratio $A_{380\ m\mu\ (o\text{-aminodiphenyl})} / A_{280\ m\mu} = 2.01 \cdot 10^{-2}$ found for fraction 180.

By subtracting the distribution curve *gck* for component D from the curve *abcde:*

$$abcde - gck = abfijde$$

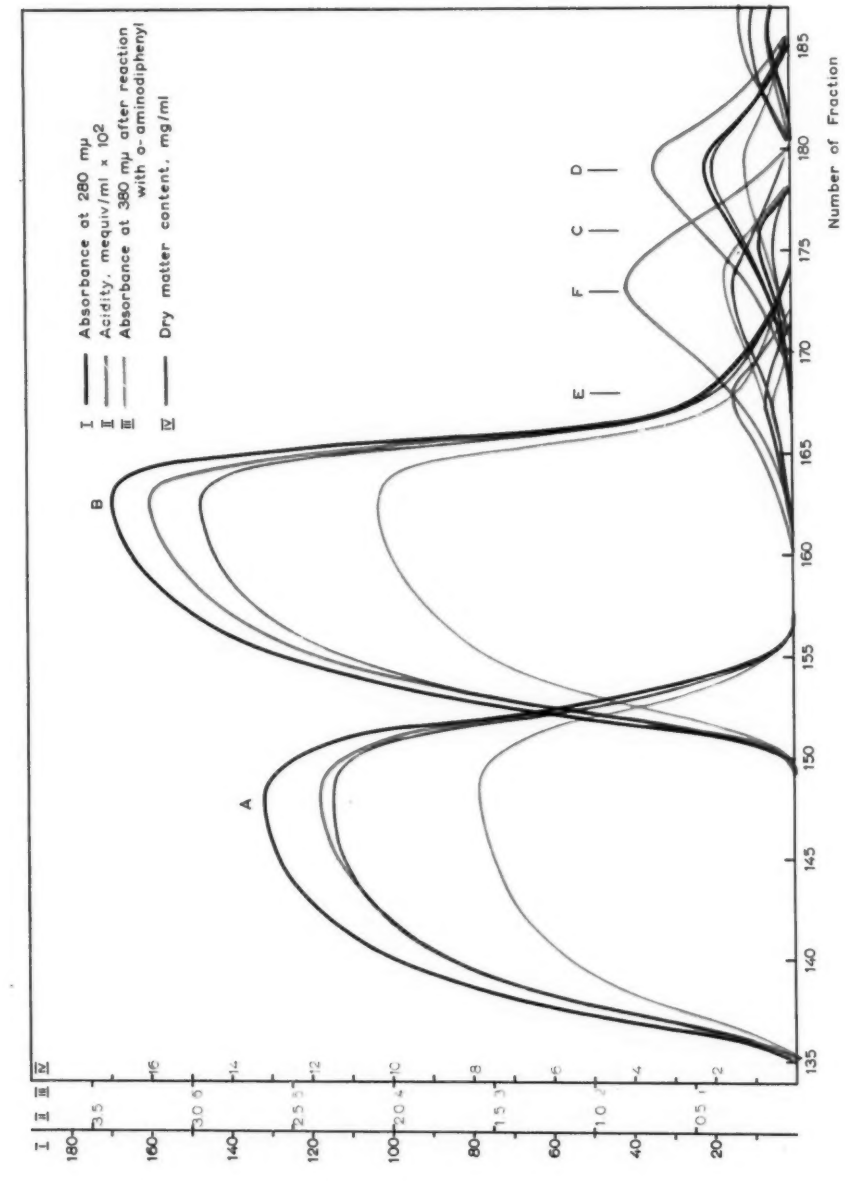


Fig. 31. The property distribution curves for the components A–F.

and drawing *af*, the distribution curve *afi* for component F was obtained. The distribution curve *ah* for component E was obtained by subtracting the curve for component F from *abfi*.

$$abfi - afi = ah$$

Results

The recorded curves in Elution Diagram 2 (Fig. 24) were divided as described above into distribution curves representing the six components A, B, C, D, E and F. These curves are drawn in Fig. 31.

Of the six components, the components A, B, C and D absorbed light of wave length 280 mμ. The components E and F did not absorb ultraviolet light, but contained acidic constituents as revealed by the ratios of absorbance to acid content in Figs. 22 and 24. All of the components except C had acid properties. The distribution curves suggest that the components A and B, which contained lignosulfonic acids, were devoid of other components in the fraction range 135—160. In the following it will be shown that this was actually the case and further that the resolution into the components A-F, although it was partly based on arbitrary assumptions, does give an idea of the compounds yielding the elution diagram shown in Fig. 24.

Investigation of the Dry Matter (Sodium Salts) in the Fractions of Fractionation 2

The dry matter content curves for components A-F of Fractionation 2 are plotted in Fig. 32.

Ultraviolet Absorption Spectra and Absorptivites ($l \cdot g^{-1} \cdot cm^{-1}$) at Wave Length 280 mμ

The ultraviolet spectra for the fractions 139, 164 and 181 containing the components A, B and D, respectively, were recorded for the sodium salts in water at pH 5—6 and in water made alkaline to pH 13 with sodium hydroxide. The recorded spectra are shown in Fig. 33.

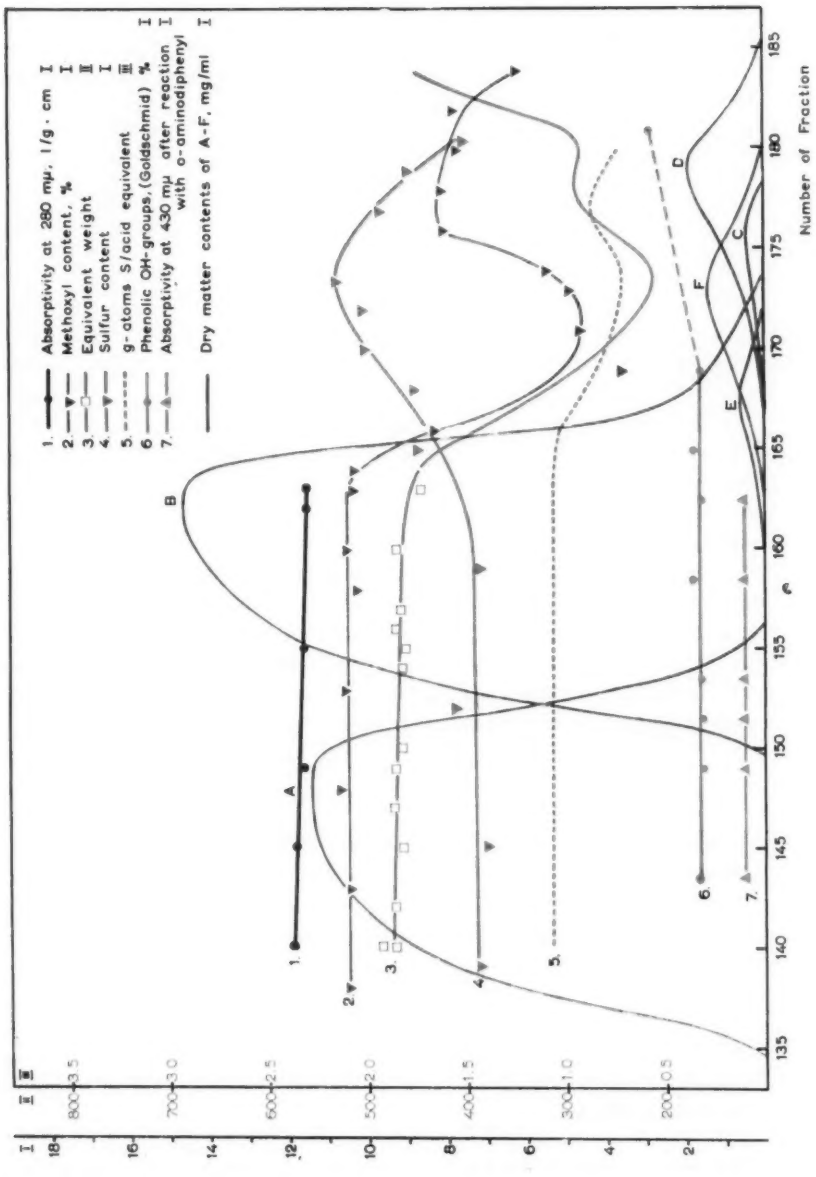


Fig. 32. Chemical and physical properties of the neutralized dry matter (sodium salts) in the fractions of Fractionation 2.

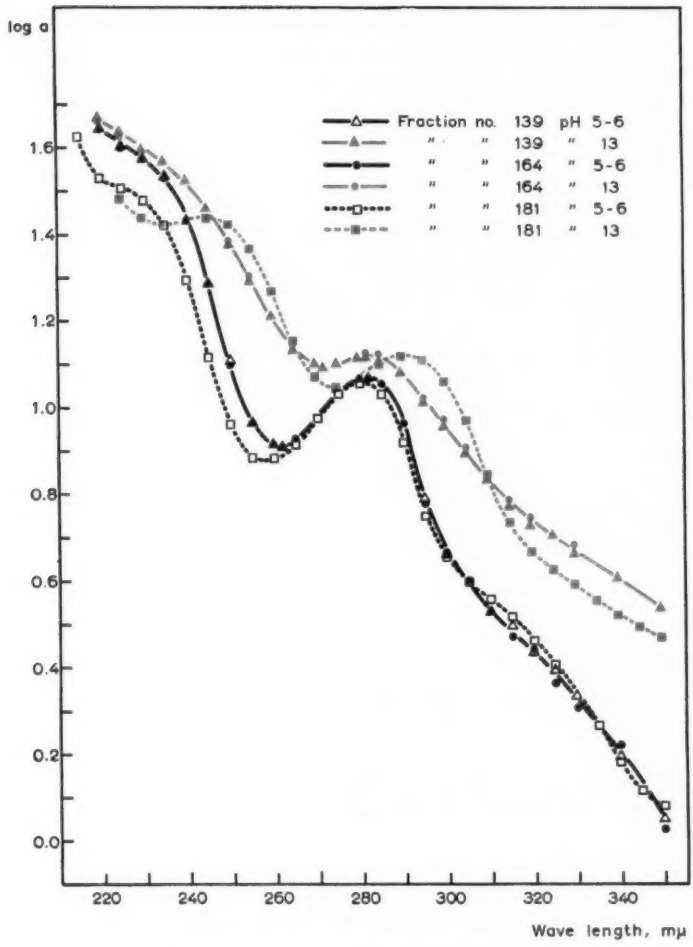


Fig. 33. Ultraviolet absorption spectra of the dry matter (sodium salts) in fractions of Fractionation 2 containing components A, B and D.

The absorptivities at 280 mμ of the dry matter (sodium salts) in fractions 140, 145, 149, 155, 159 and 162 were determined by weighing about 7 milligrams of dry matter from each fraction on a microbalance and diluting this to 50 ml with distilled water. Five dilutions of each sample were made and the absorbances measured against distilled water. The recorded absorption curves are shown in Fig. 34.

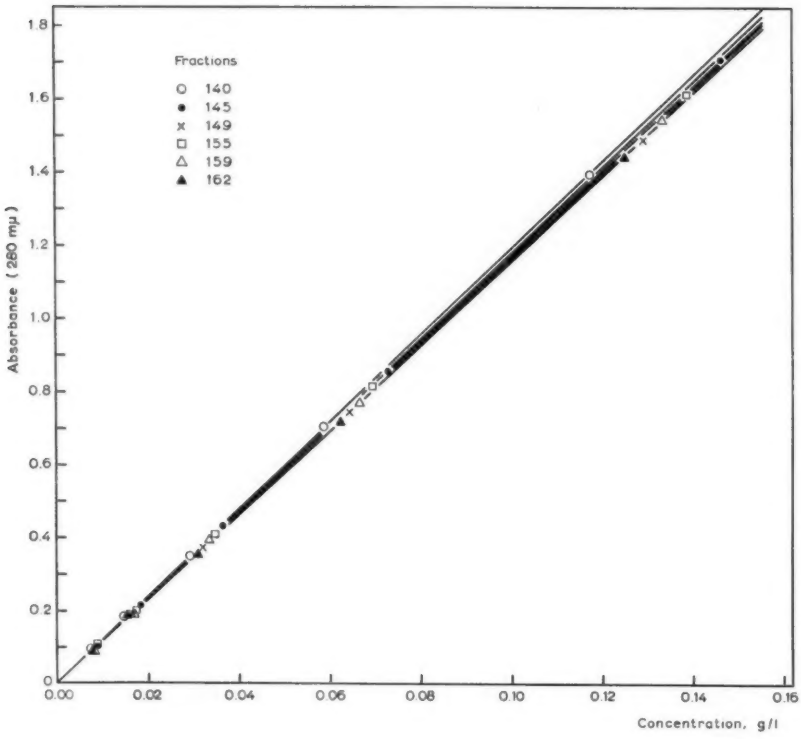


Fig. 34. The absorbance at 280 mμ as a function of concentration of the dry matter (sodium salts) for fractions of Fractionation 2 containing components A and B.

As seen from the figure, Lambert-Beer's law, $A = a \cdot c \cdot l$, where A is the absorbance, a the absorptivity, c the concentration (g/l) and l the optical path (cm) was obeyed by the data for the fractions. The lignosulfonic acids in components A and B had the same absorptivity at 280 mμ (Curve 1 in Fig. 32).

Methoxyl Contents

The methoxyl contents of the dry matter in the fractions (sodium salts) were determined by the micro Zeisel method. It was found that also the methoxyl contents were constant and equal to 10.5 per cent for both components A and B. The methoxyl content decreased in the

later fractions owing to the methoxyl-free components E and F and then increased, this increase being due to the methoxyl-containing components C and D that absorbed ultraviolet light (Curve 2 in Fig. 32).

Equivalent Weights

Samples (6—30 mg) of dry matter (sodium salts) of different fractions were weighed on a microbalance and dissolved in small amounts of distilled water free from carbon dioxide. These aqueous solutions were then passed followed by water through a Dowex-50, X-8, H^+, 50/100 mesh, column 8 cm long and 0.7 cm in diameter. The effluent was divided into three 10-ml fractions of which the middle fraction contained the acids of the sample. The first and third volume fractions were

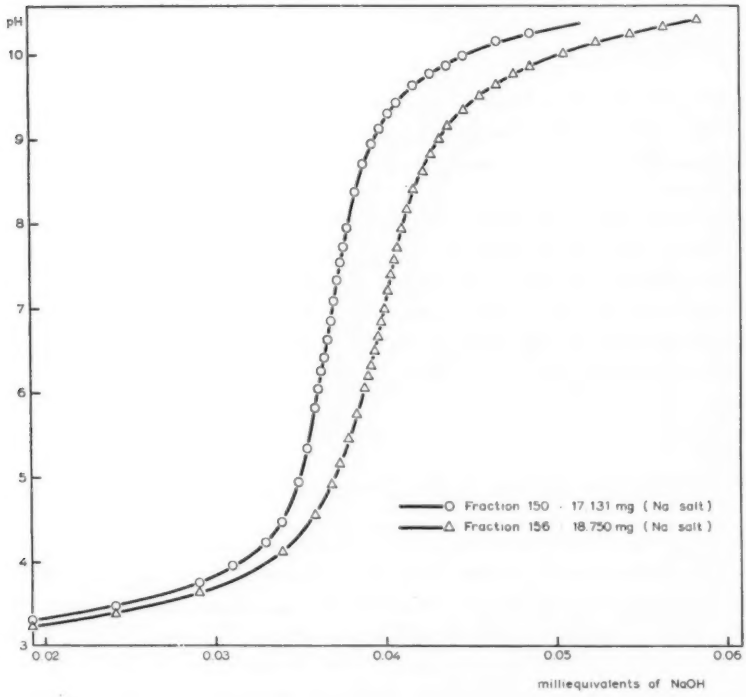


Fig. 35. Potentiometric titration of the dry matter (sodium salts) of two fractions containing components A and B.

taken as controls to determine the sodium hydroxide consumption of the solvent. The fractions containing acid were titrated potentiometrically to pH 6.8 with 0.0100 N sodium hydroxide in a nitrogen atmosphere employing a Beckman H-2 pH meter. Illustrative titration curves for the fractions 146 and 160 containing the components A and B, respectively, are shown in Fig. 35.

The titrations revealed that the equivalent weights of the components A and B were practically equal. As seen from curve 3 in Fig. 32, the equivalent weights then decreased rapidly owing to the components E and F and increased again owing to the components C and D.

It should be noted that these determinations relate only to fractions 140—163. The parts of the curve which relate to fractions 164—184 have been computed from the curves shown in Fig. 24 and are only orientative owing to the low dry matter contents of these fractions.

Sulfur Contents

Quantitative determinations of the sulfur contents of the dry matter (sodium salts) of the fractions were performed at the Alfred Bernhardt Mikroanalytisches Laboratorium im Max-Planck-Institut für Kohlenforschung, Mülheim, Germany.

Curve 4 in Fig. 32 shows that the sulfur contents of both components A and B were equal. The curve then rose rapidly owing to the high sulfur contents of the components E and F and fell again owing to the influence of the components C and D. The fraction 180 that was assumed to contain only component D had approximately the same sulfur content as components A and B.

Ratios of Sulfur Content to the Content of Acid Groups

The ratios of sulfur content to the content of acid groups were calculated from the curves 3 and 4 in Fig. 32 and are plotted as curve 5 in Fig. 32. The value of the ratio is seen to be 1.05—1.07 for the components A and B. The ratio then decreased to a minimum approximately at fraction 173. These results indicate that the lignosulfonic acid components A and B did not contain other acid groups than sulfonic acid groups (and phenolic hydroxyl groups). On the other hand, component F probably contained carboxyl groups.

Phenolic Hydroxyl Group Contents

As mentioned on p. 43, the content of phenolic hydroxyl groups can be determined from absorption spectra recorded in acid and alkaline media. The fact that the spectra for the fractions containing components A and B are identical (Fig. 33) implies that all the lignosulfonic acid fractions had the same phenolic hydroxyl group content. This was confirmed by determining by the method of Goldschmid the phenolic hydroxyl groups in the following single or combined fractions of Fractionation 2: 143—144, 148—150, 151—152, 153—154, 158—159, 161—163, 165, 169, 181. The results, which are given by curve 6 in Fig. 32, show that all the lignosulfonic acid fractions had the same phenolic hydroxyl group content, 1.53 per cent, which corresponds to 0.27 OH/MeO. However, as shown by the result for fraction 181, component D had a

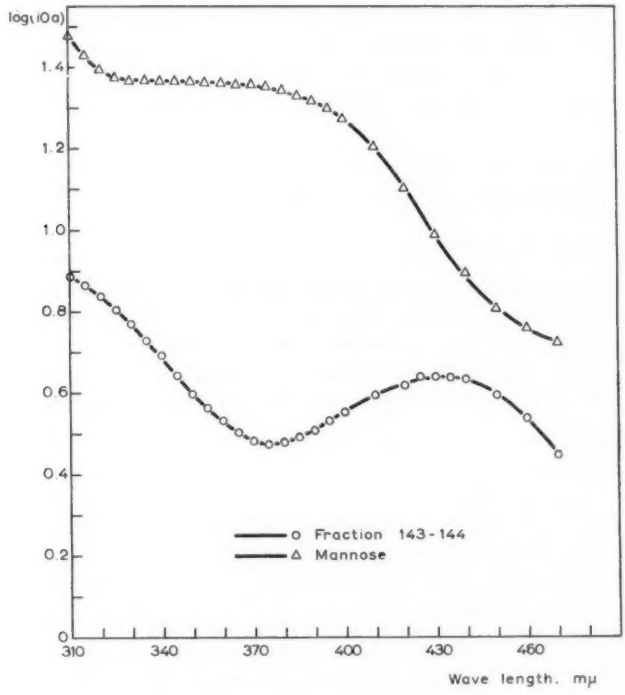


Fig. 36. Absorption spectrum of the dry matter (sodium salts) in the combined fractions 143—144 (component A) and the absorption spectrum of mannose, both after reaction with o-aminodiphenyl.

definitely higher phenolic hydroxyl group content, 2.85 per cent (compare this with the value for Braun's native lignin on p. 43).

Absorption Spectra and Absorptivities ($l \cdot g^{-1} \cdot cm^{-1}$) at 430 mμ after Reaction with o-Aminodiphenyl

As seen from Fig. 24, the fractions containing components A and B reacted with o-aminodiphenyl. The deep yellow reaction products gave an absorption maximum at about 430 mμ and a minimum at about 375 mμ. Fig. 36 shows the spectrum of the combined fractions 143—144 and the spectrum of mannose after reaction with o-aminodiphenyl.

In order to obtain sufficiently large amounts of material for the determination of the absorptivity at 430 mμ, several fractions were combined as follows: 143—144, 148—150, 151—152, 153—154, 158—159, and 162—163. The combined fractions were neutralized with sodium hydroxide to pH 6.8, the solvent evaporated and the residue dried to constant weight at 60°C. After the residues had been weighed and serially diluted with water, they were treated with o-aminodiphenyl as described on p. 89. The results of the light absorption measurements are shown in Fig. 37.

It is seen from the figure that the dry matter in all the fractions containing components A and B had the same absorptivity, 0.43 $l \cdot g^{-1} \cdot cm^{-1}$.

As mentioned on p. 43, Adler et al. have shown that the coniferylaldehyde groups of lignin react with aromatic amines to give yellow-colored solutions with an absorption maximum at approximately 445 mμ. It is thus probable that also in the present case the groups that reacted with o-aminodiphenyl were coniferylaldehyde groups, and hence that the components A and B had the same contents of coniferylaldehyde groups.

Elementary Analyses

Elementary analyses were performed on combined fractions as indicated in Table XVII. The unneutralized fractions were dried over phosphorus pentoxide in a vacuum desiccator at room temperature before the analyses. The residues 1 and 2 consisted of dark brown matter, residue 3 was reddish brown and decomposed at 95—110°C, and residue 4 was dark green and decomposed at 58—75°C.

These residues were analyzed for carbon, hydrogen, oxygen, sulfur and ash at Alfred Bernhardt Mikroanalytisches Laboratorium im Max-Planck-Institut für Kohlenforschung, Mülheim, Germany (Table XVII).

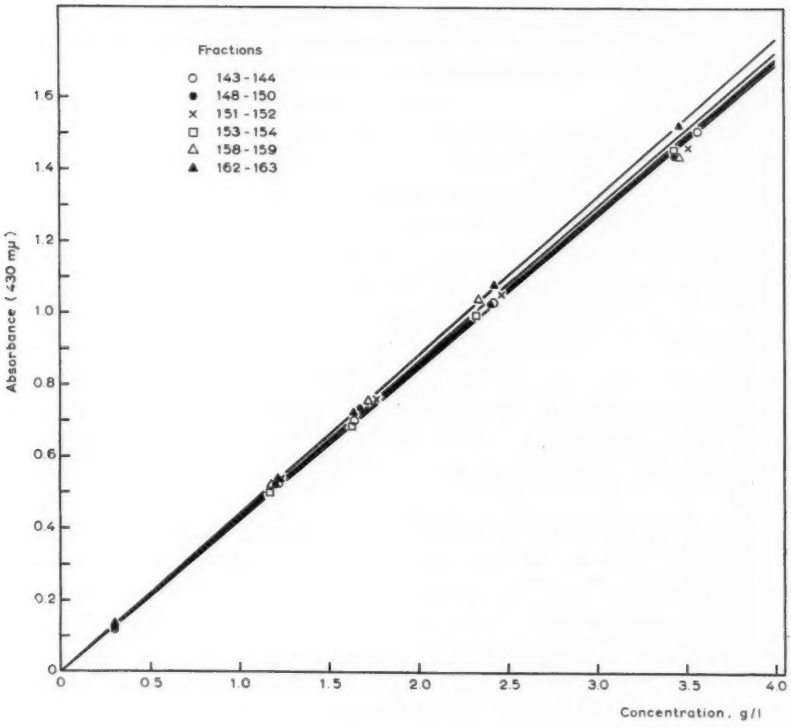


Fig. 37. The absorbance at 430 mμ after reaction with o-aminodiphenyl as a function of concentration of dry matter (sodium salts) for some combined fractions containing components A and B.

Table XVII

Elementary Composition of Unneutralized Residues of Various Fractions

Residue	Fractions	Color	C %	H %	O %	S %	Ash %	Sum %
1	146—147	brown	53.93	5.16	33.03	6.88	0.00	100.00
2	160—161	brown	54.13	5.28	34.49	6.94	0.12	100.96
3	170—176	reddish brown	48.80	5.02	36.00	7.97	1.85	99.64
4	177—184	green	55.44	5.35	31.50	7.47	0.12	99.88

When calculated on the basis of ten carbon atoms per molecule, the data yielded the following constitutional formulas:

1. $C_{10}H_{11.40}O_{4.74}S_{0.48}$
2. $C_{10}H_{11.62}O_{4.78}S_{0.48}$
3. $C_{10}H_{12.26}O_{5.54}S_{0.61}$
4. $C_{10}H_{11.50}O_{4.26}S_{0.50}$

The above gross formulas show that the components A and B, which are represented by residues 1 and 2, respectively, are identical also as far as elementary composition is concerned. The purity of the lignosulfonic acids is further indicated by the very low ash contents.

Residue 3 consisted, as shown by Fig. 31, of components B, C, D, E and F and it is therefore difficult to draw any conclusions about it. It is seen that residue 3 had the lowest carbon content and the highest oxygen and sulfur contents. Also the high ash content of the residue is noteworthy.

The analytical data for residue 4 give a formula which differs from the formulas for residues 1 and 2 by its lower oxygen content, which is 0.5 oxygen atom/phenylpropane unit lower than for the lignosulfonic components A and B. It should be noted, however, that the residue 4 contained only 70—80 per cent of component D according to Fig. 31 and furthermore that component D may also have been a mixture of several compounds.

Infrared Spectra

As mentioned on p. 73, carboxyl groups have been found in some low-molecular lignosulfonic acid preparations. If the lignosulfonic acids had contained both sulfonic acid groups and carboxyl groups the ratio of sulfur content to acidity should have been less than 1, but it was seen on p. 124 that this ratio was 1.05—1.07 for the lignosulfonic acids A and B. This implies that the lignosulfonic acids did not contain other acid groups than sulfonic acid groups (and phenolic hydroxyl groups). Samuelson et al.,[227] however, have stated that the lignosulfonic acids may also contain so-called »organic excess sulfur». The presence of

such sulfur would increase the above-mentioned ratio. It was therefore of interest to study the nature of the acid groups in the lignosulfonic acids by recording their infrared spectra.[234]

Carboxylic acids which generally exist as dimers with the carbonyl and hydroxyl groups of two carboxyl groups joined by hydrogen bonds can be identified primarily by the absorption bands due to stretching vibrations of their hydroxyl and carbonyl groups. The hydroxyl groups absorb infrared radiation with a wave number between 2700 and 2500 cm^{-1}. According to Bellamy [234] also sulfonic acid groups show some absorption in this same region. The absorption by the carbonyl group, which is stronger for carboxylic acids than for ketones, occurs in the region around 1700 cm^{-1}.

When carboxylic acids are neutralized, the absorption of the carbonyl group disappears. This is due to the resonance of the carboxylate group formed. A new band then appears at a wave number between 1610 and 1550 cm^{-1} and another at a wave number between 1400 and 1300 cm^{-1}; of these two bands the former is more prominent.

The infrared spectra of fractions containing components A and B are reproduced in Figs. 38 and 39. These spectra were recorded by the potassium bromide pellet technique with a Perkin-Elmer double beam spectrophotometer, Model 21. The samples of dry matter were obtained in three different ways:

(1) Fractions were neutralized with sodium hydroxide to pH 6.8, the solvent evaporated and the residue dried at 60°C. (2) Fractions were neutralized with sodium hydroxide to pH 4.0, the solvent evaporated, and the residue dried over phosphorus pentoxide in a vacuum desiccator at room temperature. (3) Unneutralized fractions were evaporated and dried over phosphorus pentoxide in a vacuum desiccator at room temperature.

A comparison of the spectra in Figs. 38 and 39 reveals no differences between components A and B. Both spectra show an absorption band near 1720 cm^{-1} possibly due to unconjugated carbonyl groups of saturated aliphatic acids or of α,β-unsaturated acids. As this absorption band is observed also in the spectra of the sodium salts, it must be due, at least partly, to ketonic or aldehydic carbonyl groups.

Near 2560 cm^{-1} there is an absorption band in spectra 3 in Figs. 38 and 39 due either to carboxyl groups or to sulfonic acid groups.

If the lignosulfonic acids of components A and B had contained also carboxyl groups, it would have been expected that the spectra 1 would have shown, in contrast to the spectra 2 and 3, also bands due to the ionized carboxyl group. A comparison reveals, however, that the spectra

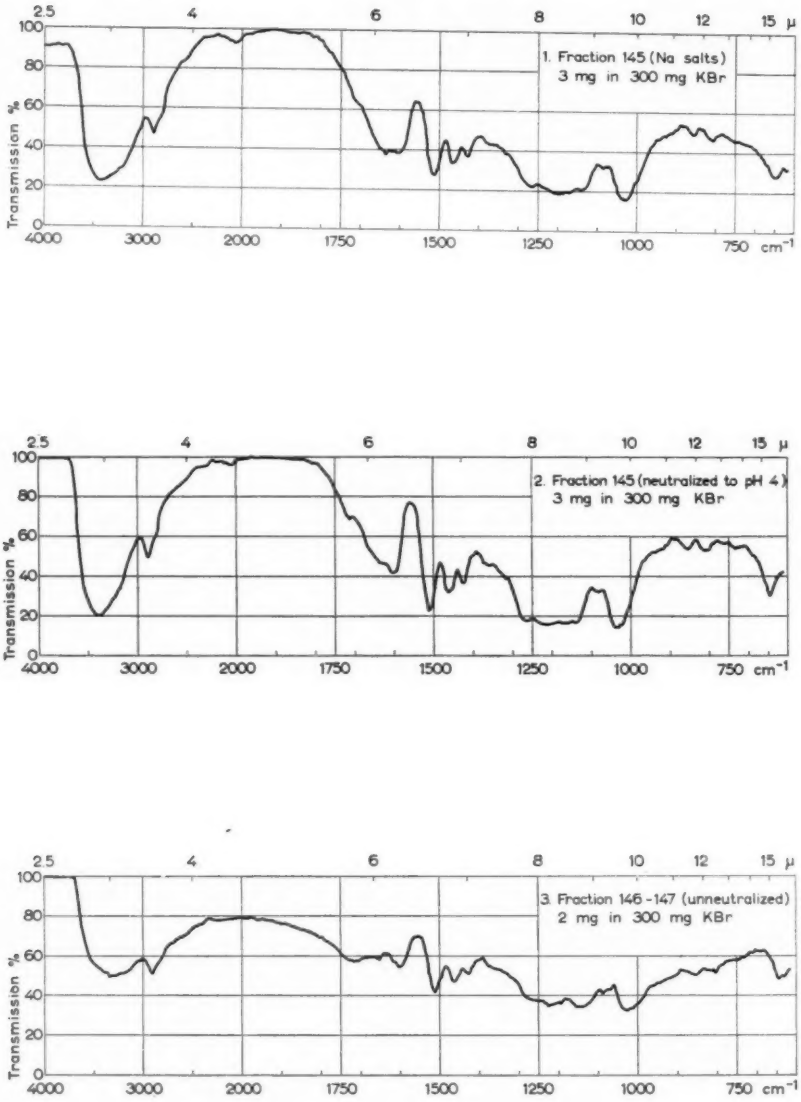


Fig. 38. **Infrared absorption spectra of dry matter from fractions containing component A.**

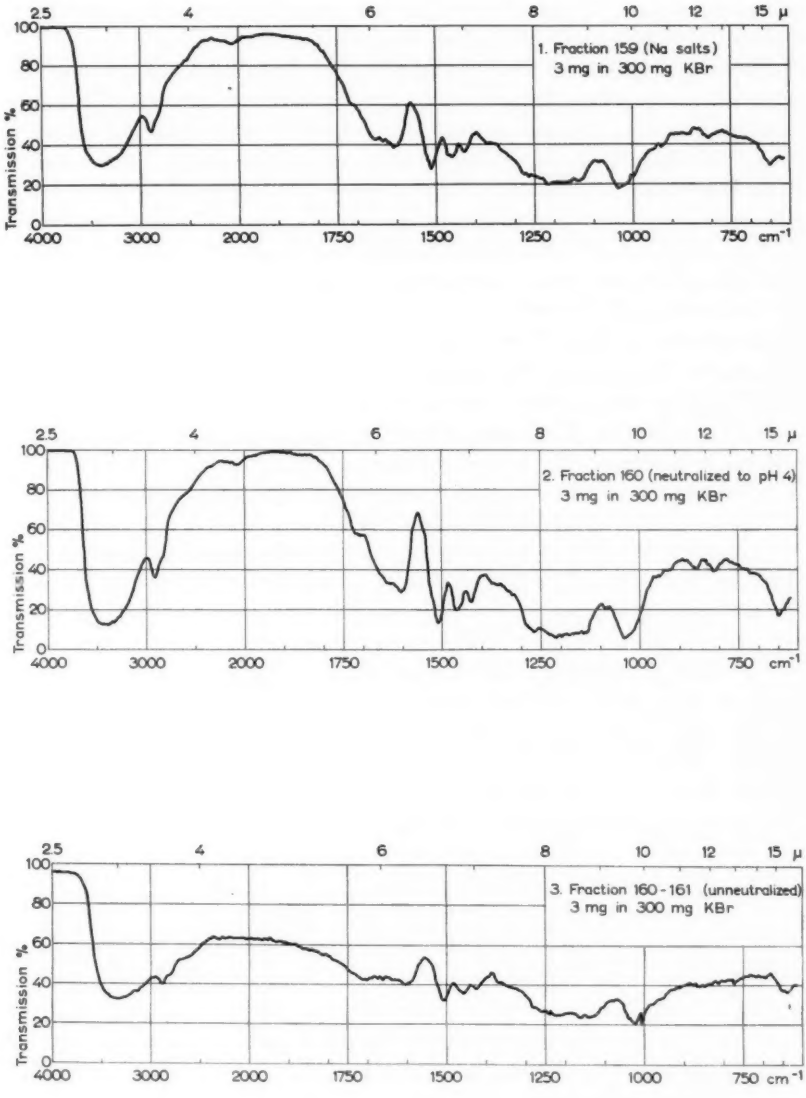


Fig. 39. Infrared absorption spectra of dry matter from fractions containing component B.

1 do not contain bands in the ranges between 1610 and 1550 cm^{-1} and between 1400 and 1300 cm^{-1} other than those in spectra 2 and 3.

Summarizing, it may be said that the absorption bands at 2560 cm^{-1} and 1720 cm^{-1} were not necessarily due to carboxyl groups, but may have been due to sulfonic acid or carbonyl groups. As the spectra of the sodium salts contained only such absorption bands as were also found in the spectra of the free acids, it is probable that the lignosulfonic acids did not contain carboxyl groups.

The spectra 3 are seen to be flatter than the spectra 1 and 2. This may have been due to a condensation of the lignosulfonic acids during the evaporation of the highly acid fractions.

The spectra in Figs. 38 and 39 closely resemble the spectrum of Björkman lignin [102] except in the region about 1175 cm^{-1} where the former spectra have a strong absorption band that is absent from the spectrum of Björkman lignin. It is probable that this absorption is caused by sulfonic acid groups.[235]

The spectrum of the dry matter (Na salts) of fraction 182 is shown in Fig. 40.

This spectrum differs from that for the sodium lignosulfonates A and B by the pronounced absorption band at 1750 cm^{-1}.

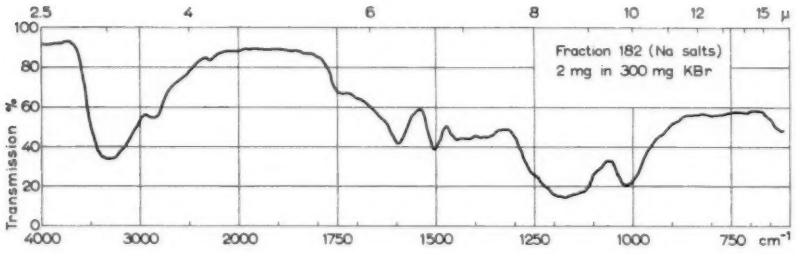


Fig. 40. Infrared absorption spectrum of the dry matter (sodium salts) from fraction 182 (component D).

Discussion of Analytical Data for Fractionation 2

The analytical results for the dry matter in the fractions of Fractionation 2 show (Fig. 32) that the division of matter among components A — F closely corresponds to the actual distribution of different compounds.

Components A and B

As the components A and B showed the same acidic properties, it seems probable that their separation was effected by differences in molecular size. The component A therefore evidently consisted of »high-molecular», and component B of »low-molecular» lignosulfonic acids.

Since the lignosulfonic acids of both components A and B showed identical chemical and physical properties, it is highly improbable that the components A and B consisted of mixtures of different compounds. It may therefore be concluded that the two components A and B were pure lignosulfonic acids.

It is furthermore obvious that the customary division of lignosulfonic acids into α- and β-lignosulfonic acids (p. 69) has no real foundation. The chemical differences that have been the basis for this differentiation have thus, with high probability, been due to impurities in the preparations. The lignosulfonic acids can probably be divided only into lignosulfonic acids with different degrees of polymerization but with the same chemical composition.

It has previously been reported that the absorptivity ($l \cdot g^{-1} \cdot cm^{-1}$) decreases with decreasing molecular weight. The absorptivity was, however, found to be the same for all the lignosulfonic acid fractions isolated from the examined spent liquor. The previously found low absorptivities of β-lignosulfonic acid samples may have been due to the presence in these samples of sulfonic acids, components E and F, that do not absorb ultraviolet light. The same sulfonic acids of high sulfur content have probably been the cause of the high sulfur contents of the earlier β-lignosulfonic acid preparations. There is hence no real difference in the degrees of sulfonation of the high- and low-molecular lignosulfonic acids in the same spent liquor.

Also the methoxyl contents were the same for both lignosulfonic acid components. That some lignosulfonic acids have lower methoxyl contents than others has thus not been observed. If a part of the methoxyl groups bound to aromatic nuclei are split off during the cooking process (p. 70), this cleavage must affect the whole of the lignin. The lignosulfonic acids A and B were found to have the same phenolic hydroxyl content per methoxyl group by the method of Goldschmid but this content was not higher than that found in unsulfonated lignin preparations.

The results of the elementary analyses do not either reveal any differences between the components A and B. The purities of the two components are indicated further by their low ash contents.

In addition to identical ultraviolet absorption spectra, the components A and B gave identical absorption spectra after reaction with o-aminodiphenyl. The identical absorptivity at 430 mμ after reaction with o-aminodiphenyl probably means that the lignosulfonic acids A and B had the same coniferyl aldehyde group content.

Curve 5 in Fig. 32 and the infrared spectra in Figs. 38 and 39 suggest that the lignosulfonic acids did not contain carboxyl groups. The carboxyl groups that have been found previously, especially in low-molecular lignosulfonic acids (p. 73), are probably to be attributed to other compounds.

The similarity of the components A and B leads to the following reflections upon the nature of coniferous lignin. As mentioned on p. 62, spent sulfite liquor contains both low-molecular lignosulfonic acids and lignosulfonic acids with molecular weights up to 100,000. If the lignin of wood is a high polymer, it must be depolymerized (hydrolyzed) during or after the sulfonation before it can diffuse into the cooking liquor. At the same time or later, the sulfonated and hydrolyzed lignin must undergo condensation to lignosulfonic acids of the observed molecular sizes.

If the polymeric protolignin of wood is randomly built of different structural units, for example, as suggested on p. 37, one would expect a difference in reactivity between the various hydrolyzable bonds and the different sufonatable groups. One would hence expect that the various structural elements would be hydrolyzed and sulfonated in, at least to some extent, a definite sequence. Likewise certain structural units would be expected to exhibit a greater affinity to each other in the formation of high-molecular lignosulfonic acids.

The reactions, hydrolysis, sulfonation and condensation, of lignin during a sulfite cook should therefore lead to a rearrangement of the original randomly built high polymers to more or less ordered different lignosulfonic acids. As an example, it might be supposed that the guaiacylglycerol-β-aryl ether structure becomes enriched in the high-molecular lignosulfonic acids, whereas the dehydrodiconiferyl alcohol structures would predominate in the low-molecular lignosulfonic acids. The difference in the ease with which these structures are sulfonated should then have been reflected in the properties of the components A and B separated by ion exclusion.

If the above reactions of lignin were to take place actually during a sulfite cook, the similarity of the components A and B could be more easily explained by assuming that the protolignin of wood is an ordered high polymer formed from only a small number of structural units.

Hydrolysis and sulfonation would then liberate identical groups of units from the lignin. These structures would then combine to form larger aggregates which, as in the case of components A and B, differ from each other primarily in molecular weight.

Component D

The analytical results show that the component D contained methoxyl groups and sulfur. Further, it absorbed ultraviolet light and had an elementary composition resembling that of the lignosulfonic acids. One may then ask whether component D, like components A and B, is composed of lignosulfonic acids?

The ultraviolet spectra of fractions 139, 164 and 181 representing A, B and D are shown in Fig. 33. It is seen that fractions 139 and 164 have identical spectra in both acid and alkaline media. It is seen further that no shift of the absorption maximum at 280 mμ occurs when the solutions of lignosulfonic acids A and B are made alkaline. The spectrum of fraction 181 in acid medium is similar to the spectra of the lignosulfonic acids A and B, but the spectrum in alkaline medium is, however, quite different. The absorption maximum which is at 280 mμ in acid and neutral solution has shifted about 10 mμ to longer wave lengths and there is a new absorption maximum at 245 mμ.

It is obvious from the ultraviolet spectra in alkaline medium that component D does not consist of true lignosulfonic acids. However, when Figs. 6, 8 and 33 are compared, it is seen that component D gives spectra in both acid and alkaline media that are similar to the spectra of Brauns' native lignin, sulfonated Brauns' native lignin and lignans such as α-conidendrin.

As mentioned on p. 77, Freudenberg and Knof have stated that hydroxymatairesinol, allo-hydroxymatairesinol and α-conidendrin are the three major lignans in spruce wood (Picea excelsa). Of these, only α-conidendrin has been so far isolated from spent sulfite liquor. Hydroxymatairesinol **(91)** differs from α-conidendrin **(92)** in having a hydroxyl group on the alpha carbon atom. As the former lignan is probably therefore sulfonated during the sulfite cook, it is possible that component D was at least partly composed of the lignansulfonic acid hydroxymatairesinolsulfonic acid. Hydroxymatairesinol also contains a γ-lactone ring. According to Bellamy,[234] γ-lactones strongly absorb light in the wave number region 1800—1740 cm^{-1}. In accordance with this, α-conidendrin has a strong absorption band at 1750 cm^{-1}.[236]

The infrared spectra of fractions containing components A, B and D (Figs. 38, 39 and 40) show that D, unlike A and B, strongly absorbed light of wave number 1750 cm^{-1}. This supports the view that D contained hydroxymatairesinolsulfonic acid.

The elementary constitution of hydroxymatairesinolsulfonic acid computed on the basis of ten carbon atoms:

$$C_{10.00}H_{11.00}O_{4.50}S_{0.50}$$

does not differ essentially from the constitution found for the matter in the combined fractions 177—184 (p. 128). It is stressed, however, that although it was possible to deduce that the components A and B consisted of pure and uniformly built lignosulfonic acids, it cannot be decided whether or not component D consisted of one compound or of several compounds with the same distribution coefficient. It is quite possible that component D contained also other sulfonated lignans.

Components E and F

According to Fig. 31, components E and F contained acids that do not absorb ultraviolet light but are highly reactive towards o-aminodiphenyl. This latter points to the presence of carbonyl groups.

The analyses of the dry matter of the fractions containing components E and F clarify the situation further. Neither E nor F contained methoxyl groups. The sulfur contents, however, were much higher than those of lignosulfonic acids. Thus, for instance, component F (sodium salts) contained about 16 per cent sulfur, which is more than twice the sulfur content of sodium lignosulfonates. The sulfur was probably present in sulfonic acid groups. In this connection it should be noted that a part of the sulfur in the original solution L_4 was present as loosely bound sulfur dioxide (Table X and p. 80). The proportion of sulfur present as loosely bound sulfur dioxide in components E and F was not determined.

As seen from curve 5 in Fig. 32, component F possibly contained also carboxyl groups in addition to sulfonic acid groups. The mean equivalent weight of component F was between 150 and 200.

If the examined spent liquor contained carbohydrate sulfonic acids which are assumed to exist in spent sulfite liquors (p. 71), it is highly probable that these were present in components E and F. The analytical data do not deny this possibility.

Component C

Fig. 31 reveals that component C was neutral and absorbed ultraviolet light, but did not react with o-aminodiphenyl. The analyses show further that in all probability this component had a high methoxyl content (Fig. 32). It is possible that component C consisted of α-conidendrin and, perhaps, other unsulfonated lignans.

The Quantitative Distribution of Components A—F in Solution L_4

Table XVIII shows the dry matter contents, acid equivalents, absorbances at 280 mμ and the absorbances at 380 mμ after reaction with o-aminodiphenyl of components A—F, all calculated as percentages of the corresponding values found for solution L_4.

Table XVIII

Analytical Data for Components A—F Expressed as Percentages of the Corresponding Values for the Matter in Solution L_4

Component	Dry Matter	Acid Equivalents	Absorbance at 280 mμ	Absorbance at 380 mμ (o-aminodiphenyl)
	%	%	%	%
A	23.7	22.6	39.7	8.8
B	28.6	26.8	46.3	10.9
C	0.4	0.0	0.9	0.0
D	2.2	3.4	3.7	0.7
E	0.6	1.2	0.0	0.2
F	1.9	4.8	0.0	1.1
Total	57.4	58.8	90.6	21.7
Percentage of the value found for Fraction Group I of Fractionation 1	95.7	99.7	96.2	100.5

THE QUANTITATIVE DISTRIBUTION OF THE DRY MATTER IN SOLUTION L_4

A summary of the dry matter contents of fractions of Fractionations 1 and 2 is presented in Table XIX.

Table XIX
Distribution of Dry Matter in Solution L_4

		percentage
Fraction Group I		
Component A: lignosulfonic acids, probably »high-molecular»	23.7	
» B: » » , » »low-molecular»	28.6	
» C: α-conidendrin?	0.4	
» D: possibly hydroxymataresinolsulfonic acid	2.2	
» E: carbohydrate sulfonic acids?	0.6	
» F: » » » ?	1.9	
Substances not investigated	2.6	60.0
Fraction Group II		
One main component, in all probability O-(4-O-methyl-α-D-glucosyluronic acid)-(1→2)-D-xylose. Furthermore compounds giving glucose and mannose on acid hydrolysis		2.5
Fraction Group III		
Main component probably glucuronic acid. Small amounts of a polysaccharide containing glucose and mannose and probably small amounts of methoxyl-containing nonaromatic compounds		1.0
Fraction Group IV		
Four polysaccharides giving the following monosaccharides on acid hydrolysis:		
1. Glucose, mannose, galactose		
2. Glucose, mannose		
3. Mannose, glucose		
4. Xylose		3.2
Fraction Group V		
D-Glucose	2.6	
D-Xylose	4.6	
D-Mannose	11.0	
D-Galactose	2.6	
L-Arabinose	0.9	
Substances not investigated, possibly aldonic acids	4.4	26.1
Fraction Group VI		
Main component: acetic acid. Small amounts of other low-molecular compounds		5.7
	Total	98.5 %

SUMMARY

The morphological structure and chemical composition of spruce wood and the reactions of the various constituents during a sulfite cook are outlined in the first two chapters.

After a brief description of ion exclusion and previous attempts to fractionate spent sulfite liquor by this method, the fractionation by ion exclusion of a spent liquor from an industrial sulfite cook of spruce yielding a strong pulp is described.

The spent sulfite liquor was passed first through a cation exchange resin to liberate the acids. The directly titratable and the greater part of the loosely bound sulfur dioxide was then removed with a stream of nitrogen gas. After neutralization with barium hydroxide and centrifugation, the solution was evaporated to half its original volume. The original spent sulfite liquor, the evaporated solution and other solutions in which the volatile compounds were collected were analyzed. In connection with the analyses, it was established that an iodometric titration of the spent sulfite liquor, for example, by the TAPPI method 629-m 48, cannot be relied upon to give correct values for the directly titratable sulfur dioxide in spent sulfite liquors because also the thiosulfate in the liquor reacts with iodine.

A measured volume of the evaporated spent sulfite liquor was added onto a column of a strong cation exchange resin in the hydrogen form and eluted with water. The column was 4 meters long and 4.2 cm in diameter. The effluent was divided into 550 fractions, each about 10 ml in volume. The fractions were analyzed and the analytical data plotted to give elution diagrams.

The analyses showed that the compounds that absorb ultraviolet light (lignosulfonic acids and other aromatic components in the spent liquor) were eluted as two peaks in the fractions 156—200. A comparison of the curves plotting absorbances at 280 mμ and acidities of the fractions revealed that these contained also acids that did not absorb ultraviolet light.

After being treated with o-aminodiphenyl (which reacts with the carbonyl groups of carbohydrates and the coniferylaldehyde groups of

lignosulfonic acids), the fractions 240—280 relatively strongly absorbed light of wave length 380 mμ. The analytical data showed that the main component in these fractions was probably O-(4-O-methyl-α-D-glucosyluronic acid)-(1 → 2)-D-xylose.

The fractions 301—329 contained as the major component a compound which in all probability was glucuronic acid and an additional component composed of glucose and mannose.

The fractions 330—362 were found to contain four polysaccharide components that reacted with aniline oxalate. These polysaccharides were composed of (1) glucose, mannose and galactose, (2) mannose and glucose, (3) glucose and mannose, and (4) xylose.

The fractions 360—449 contained monosaccharides. The monosaccharides had become partly separated so that the fractions 360—384 contained glucose, the fractions 370—405 xylose, mannose and galactose, and the fractions 404—449 arabinose. The amounts of various monosaccharides in the fractions were determined. The fractions contained in addition appreciable amounts of other compounds, probably aldonic acids.

The acetic acid in the spent sulfite liquor was concentrated in the fractions on both sides of fraction 491. The fractions 480 and 510 contained also small amounts of neutral compounds; the neutral compounds of fraction 480 gave a rose-colored product with o-aminodiphenyl.

The total dry matter content of all fractions represented 98.5 per cent of the dry matter in the original sample of organic solutes from the spent sulfite liquor.

In order to achieve a better separation of the lignosulfonic acids and the acids that did not absorb ultraviolet light, a new fractionation was made at a lower flow rate. Only the fractions 134—200 that contained compounds which absorbed ultraviolet light and the acids associated with them that did not absorb ultraviolet light were collected.

The elution diagram revealed that the lignosulfonic acids had separated into two peaks which were designated A and B. Two lower peaks contained components C and D which also absorbed utraviolet light. Two components, E and F, which did not absorb ultraviolet light were detected by graphic analysis of the property distribution curves.

The analytical data for the dry matter in the various fractions showed that the components A and B contained compounds with properties typical of the so-called α-lignosulfonic acids. The compounds of both peaks had identical properties and it was concluded that the lignosulfonic acids had been completely isolated from other compounds. It

was established further that the spent sulfite liquor did not contain so-called β-lignosulfonic acids which are characterized by low methoxyl and high sulfur contents. The properties of β-lignosulfonic acids that distinguish them from α-lignosulfonic acids are probably due to the fact that the former acids represent a mixture of lignosulfonic acids and other methoxyl-free compounds of high sulfur content. The components E and F contained compounds of the latter type. The sodium salt of component F was found to contain about 16 per cent sulfur, which is more than twice the sulfur content of sodium lignosulfonates.

The component D gave analytical data which closely resembled those of the lignosulfonic acids. Although the ultraviolet absorption spectra of component D and the lignosulfonic acids A and B were very much alike in both acid and neutral media, the spectrum of component D in alkaline solution differed greatly from the spectrum of the lignosulfonic acids in this medium but closely resembled the spectra of Brauns' native lignin, sulfonated Brauns' native lignin and α-conidendrin. The infrared spectrum of component D gave an indication that the latter component contains a lactone ring of the type present in α-conidendrin. As component D, however, was found by elementary analysis to contain about 0.5 atom of sulfur per phenylpropane unit, it could not be α-conidendrin. Moreover, spruce wood contains much more hydroxymatairesinol than α-conidendrin. Both these lignans are γ-lactones, but the former has in addition a hydroxyl group in the α-position. As the former may become sulfonated at the α-carbon atom during a sulfite cook, it is not impossible that the component D consisted of hydroxymatairesinolsulfonic acid.

The quantitative distribution of the dry matter in the prefractionated spent sulfite liquor is summarized in a table (p. 138).

REFERENCES

1. Casey, J. P., »Pulp and Paper», Vol. I, Interscience Publishers, New York, 1952, p. 128.
2. Nikitin, N. I., »Die Chemie des Holzes», Akademie-Verlag, Berlin, 1955, p. 476.
3. Hägglund, E., »Chemistry of Wood», Academic Press, New York, 1951, p. 431.
4. Härtel, O., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 97.
5. Trendelenburg, R., and Mayer-Wegelin, H., »Das Holz als Rohstoff», Zweite Auflage, Carl Hansner Verlag, München, 1955, p. 152.
6. Kerr, T., and Bailey, J. W., *J. Arnold Arboretum (Harvard Univ.)* **15**, 327 (1934).
7. Meier, H., *Holz Roh- u. Werkstoff* **13**, 323 (1955).
8. Meier, H., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 181.
9. Mühlethaler, K., *Z. Zellforsch. u. mikroskop. Anat.* **38**, 299 (1953).
10. Asunmaa, S., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 182.
11. Meier, H., *Svensk Papperstidn.* **61**, no. 18 B, 43 (1958).
12. Bailey, A. J., *Ind. Eng. Chem. Anal. Ed.* **8**, 52, 389 (1936).
13. Lange, P. W., *Svensk Papperstidn.* **57**, 525 (1954).
14. Treiber, E., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 199.
15. Treiber, E., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 166.
16. Jayme, G., and Hunger, G., *Holz Roh- u. Werkstoff* **13**, 212 (1955).
17. Asunmaa, S., and Lange, P. W., *Svensk Papperstidn.* **57**, 501 (1954).
18. Meier, H., and Yllner, S., *Svensk Papperstidn.* **59**, 395 (1956).
19. Frey-Wyssling, A., »Die pflanzliche Zellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1959, p. 35.
20. Meier, H., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957 p. 221.
21. Wardrop, A. B., *Tappi*, **40**, 225 (1957).
22. Gustafsson, Ch., *Paperi ja Puu — Papper och Trä* **38**, 383 (1956).
23. Hägglund, E., »Chemistry of Wood», Academic Press, New York, 1951, p. 356.
24. Casey, J. P., »Pulp and Paper», Vol. I, Interscience Publishers, New York, 1952, p. 74.
25. Frey-Wyssling, A., »Die pflanzliche Zellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1959, p. 15, 114.
26. Sundman, J., *Pappers- och Trävarutidskr. Finland* **29**, 113 (1947).
27. Treiber, E., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 224.

28. Sundman, J., Saarnio, J., and Gustafsson, Ch., *Pappers- och Trävarutidskr. Finland* **31**, 467 (1949).
29. Jayme, G., and Hahn, G., *Holzforschung* **14**, 52 (1960).
30. Gustafsson, Ch., Sundman, J., Pettersson, S., and Lindh, T., *Paperi ja Puu — Papper och Trä* **33 B**, 300 (1951).
31. Hägglund, E., and Sandelin, O., *Svensk Kem. Tidskr.* **46**, 83 (1934).
32. Lindberg, B., and Meier, H., *Svensk Papperstidn.* **60**, 785 (1957).
33. Croon, I., and Lindberg, B., *Acta Chem. Scand.* **12**, 453 (1958).
34. Lindberg, B., *Svensk Papperstidn.* **61**, no. 18 B, 85 (1958).
35. Meier, H., *Acta Chem. Scand.* **14**, 749 (1960).
36. Aspinall, G. O., and Carter, M. E., *J. Chem. Soc.* **1956**, 3744.
37. Aspinall, G. O., in Wolfrom, M. L., »Advances in Carbohydrate Chemistry», Vol. 14, Academic Press, New York, London, 1959, p. 443.
38. Saarnio, J., Dissertation, 1956, University of Helsinki.
39. Sundman, J., *Paperi ja Puu — Papper och Trä* **32** B, 267 (1950).
40. Hägglund, E., and Klingstedt, F. W., *Cellulosechemie* **5**, 57 (1924).
41. Gustafsson, Ch., Unpublished results.
42. Roschier, R. H., and Hyvärinen, S., *Paperi ja Puu — Papper och Trä* **35**, 1 (1953).
43. Roschier, R. H., Hyvärinen, S., and Ahola, A., *Paperi ja Puu — Papper och Trä* **35**, 181 (1953).
44. Roschier, R. H., and Eskola, K., *Paperi ja Puu — Papper och Trä* **37**, 399 (1955).
45. Shaw, A. C., *Pulp Paper Mag. Can.* **57**, no. 1, 95 (1956).
46. Hägglund, E., »Chemistry of Wood», Academic Press, New York, 1951, p. 456.
47. Klingstedt, F. W., *Pappers- och Trävarutidskr. Finland* **19**, 613, 648 (1937).
48. Timell, T. E., *Svensk Papperstidn.* **60**, 762 (1957).
49. Stockman, L., *Svensk Papperstidn.* **54**, 621 (1951).
50. Ahlén, L., and Samuelson, O., *Svensk Papperstidn.* **58**, 421 (1595).
51. Bergström, H., *Papier-Fabr.* **10**, 677 (1912).
52. Hägglund, E., Heiwinkel, H., and Bergek, T., *Cellulosechemie* **21**, 108 (1943).
53. Hägglund, E., »Chemistry of Wood», Academic Press, New York, 1951, p. 430.
54. Samuelson, O., Ljungqvist, K. J., and Parck, C., *Svensk Papperstidn.* **61**, 1043 (1958).
55. Hägglund, E., and Johnson, T., *Pappers- och Trävarutidskr. Finland* **11**, 176 (1929).
56. Samuelson, O., *Svensk Papperstidn.* **61**, 531 (1958).
57. Erdtman, H., *Svensk Papperstidn.* **45**, 374 (1942).
58. Erdtman, H., Ericson, P., and Hägglund, E., *Svensk Papperstidn.* **46**, 121 (1943).
59. Tsypkina, M. N., and Balashova, I. M., *Zhur. Priklad. Khim.* **32**, 166 (1959).
60. Kosilova, E. I., and Nepenin, N. N., *Trudy Leningrad. Lesotechn. Akad.* **87**, 23 (1959).
61. Hägglund, E., Heiwinkel, H., and Bergek, T., J. prakt. Chem. **162**, 2 (1943).
62. Adler, E., *Svensk Papperstidn.* **49**, 339 (1946).
63. Hägglund, E., Johnson, T., and Urban, H., Ber. **63**, 1387 (1930).
64. Yllner, S., *Acta Chem. Scand.* **10**, 1251 (1956).
65. Adler, E., *Svensk Papperstidn.* **50**, no. 11 B, 9 (1947).
66. Fischler, F., *Z. physiol. Chem. Hoppe-Seyler's* **165**, 53 (1927).
67. Enders, C., *Biochem. Z.* **312**, 349 (1942); *Naturwissenschaften* **31**, 92 (1943).
68. Routala, O., and Yli-Jama, O., *Pappers- och Trävarutidskr. Finland* **18**, 342 (1936).

69. Routala, O., and Parpola, A., *Suomen Kemistilehti* **9 B**, 18 (1936).
70. Routala, O., and Vauhkonen, T., *Suomen Kemistilehti* **10 B**, 2 (1937).
71. Gadd, G. O., *Pappers- och Trävarutidskr. Finland* **28**, no. 7 A, 61 (1946).
72. Nokihara, E., Tuttle, M. J., Felicetta, V. F., and McCarthy, J. L., *J. Am. Chem. Soc.* **79**, 4495 (1957).
73. Marx, M., and Schulz, G. V., *Das Papier* **9**, 13 (1955).
74. Tiemann, F., *Ber.* **8**, 1127 (1875).
75. Tiemann, F., and Mendelsohn, B., *Ber.* **8**, 1136 (1875).
76. Klason, P., *Svensk Kem. Tidskr.* **9**, 133 (1897).
77. Cousin, H., and Hérissey, H., *Compt. Rend.* **146**, 1413 (1908); **147**, 247 (1908).
78. Erdtman, H., Ann. **503**, 283 (1933).
79. Freudenberg, K., Reznik, H., Fuchs, W., and Reichert, M., *Naturwissenschaften* **42**, 29 (1955).
80. Freudenberg, K., and Niedercorn, F., *Chem. Ber.* **91**, 591 (1958).
81. Freudenberg, K., Harkin, J., Reichert, M., and Fukuzumi, T., *Chem. Ber.* **91**, 581 (1958).
82. Frey-Wyssling, A., »Die pflanzliche Zellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1959, p. 38.
83. Freudenberg, K., *Chem. Ber.* **92**, LXXXIX (1959).
84. Adler, E., in Kratzl, K., and Billek, G., »Biochemistry of Wood», Proceedings of the Fourth International Congress of Biochemistry, Vienna, 1958, Vol. II, Pergamon Press, London, New York, Paris, Los Angeles, 1959, p. 137.
85. Freudenberg, K., Seib, K., and Dall, K., *Chem. Ber.* **92**, 807 (1959).
86. Freudenberg, K., and Grion, G., *Chem. Ber.* **92**, 1355 (1959).
87. Freudenberg, K., and Sakakibara, A., *Ann.* **623**, 129 (1959).
88. Björkman, A., *Nature* **174**, 1057 (1954).
89. Björkman, A., *Svensk Papperstidn.* **59**, 477 (1956).
90. Björkman, A., and Person, B., *Svensk Papperstidn.* **60**, 158 (1957).
91. Adler, E., *Ind. Eng. Chem.* **49**, 1377 (1957).
92. Freudenberg, K., and Dall, K., *Naturwissenschaften* **42**, 606 (1955).
93. Enkvist, T., Alm, B., and Holm, B., *Paperi ja Puu — Papper och Trä* **38**, 1 (1956).
94. Adler, E., Hernestam, S., and Walldén, I., *Svensk Papperstidn.* **61**, no. 18 B, 641 (1958).
95. Aulin-Erdtman, G., *Svensk Kem. Tidskr.* **70**, 4 (1958).
96. Goldschmid, O., *Anal. Chem.* **26**, 1421 (1954).
97. Lindberg, J. J., and Enkvist, T., *Suomen Kemistilehti* **28 B**, 23 (1955).
98. Adler, E., Björkqvist, K. J., and Häggroth, S., *Acta Chem. Scand.* **2**, 93 (1948).
99. Adler, E., and Ellmer, L., *Acta Chem. Scand.* **2**, 839 (1948).
100. Adler, E., and Gierer, J., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 457.
101. Adler, E., in Hägglund, E., »Chemistry of Wood», Academic Press, New York, 1951, p. 190.
102. Adler, E., and Marton, J., *Acta Chem. Scand.* **13**, 75 (1959).
103. Adler, E., and Walldén, I., Unpublished results, reported by Adler, E., and Gierer, J., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 453.
104. Adler, E., and Gierer, J., *Acta Chem. Scand.* **9**, 84 (1955).
105. Gierer, J., *Acta Chem. Scand.* **8**, 1319 (1954).

106. Gierer, J., *Chem. Ber.* **89**, 257 (1956).
107. West, E., MacInnes, A. S., and Hibbert, H., *J. Am. Chem. Soc.* **65**, 1187 (1943).
108. Hibbert, H., *Ann. Rev. Biochem.* **11**, 183 (1942).
109. Gardner, J. A. F., *Can. J. Chem.* **32**, 532 (1954).
110. Freudenberg, K., and Dietrich, G., *Ann.* **563**, 146 (1949).
111. Richtzenhain, H., *Svensk Papperstidn.* **53**, 644 (1950).
112. Bower, J. R., Cooke, L. M., and Hibbert, H.,*J. Am. Chem. Soc.* **65**, 1192 (1943.
113. Godard, H. P., McCarthy, J. L., and Hibbert, H., *J. Am. Chem. Soc.* **63**, 3061 (1941).
114. Harris, E. E., D'Ianni, J., and Adkins, H., *J. Am. Chem. Soc.* **60**, 1467 (1938).
115. Schorygina, N., Kefeli, T., and Semechkina, A. F., *Doklady Akad. Nauk. S.S.S.R.* **64**, 689 (1949).
116. Nikitin, N. I., »Die Chemie des Holzes», Akademie-Verlag, Berlin, 1955, p. 301.
117. Adler, E., and Gierer, J., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 472.
118. Adler, E., and Yllner, S., *Acta Chem. Scand.* **7**, 570 (1953).
119. Erdtman, H., and Leopold, B., *Acta Chem. Scand.* **3**, 1358 (1949).
120. Adler, E., Lindgren, B. O., and Saedén, U., *Svensk Papperstidn.* **55**, 245 (1952).
121. Adler, E., Pepper, J. M., and Eriksoo, E., *Ind. Eng. Chem.* **49**, 1392 (1957).
122. Freudenberg, K., in Zechmeister, L., »Progress in the Chemistry of Organic Natural Products», Vol. XI, Springer Verlag, Vienna, 1954, p. 51.
123. Freudenberg, K., and Bittner, F., *Chem. Ber.* **86**, 155 (1953).
124. Freudenberg, K., and Lautsch, W., *Naturwissenschaften* **27**, 227 (1939).
125. Freudenberg, K., *Angew. Chem.* **52**, 362 (1939).
126. Freudenberg, K., Lautsch, W., and Gugler, K., *Ber.* **73**, 167 (1940).
127. Leopold, B., *Acta Chem. Scand.* **6**, 38 (1952).
128. Leopold, B., *Svensk Kem. Tidskr.* **64**, 18 (1952).
129. Lautsch, W., *Cellulosechemie* **19**, 69 (1941).
130. Leopold, B., *Acta Chem. Scand.* **4**, 1523 (1950).
131. Pew, J. C., *J. Am. Chem. Soc.* **77**, 2831 (1955).
132. Richtzenhain, H., *Svensk Papperstidn.* **53**, 644 (1950).
133. Freudenberg, K., and Niedercorn, F., *Chem. Ber.* **89**, 2168 (1956).
134. Aulin-Erdtman, G., *Svensk Papperstidn.* **55**, 745 (1952).
135. Pearl, J. A., and Beyer, D. L., *Tappi* **39**, 171 (1956).
136. Freudenberg, K., *Angew. Chem.* **68**, 508 (1956).
137. Freudenberg, K., and Ahlhaus, O., *Monatsh.* **87**, 1 (1956).
138. Schorygina, N. N., Kefeli, T., and Semechkina, A. F., *Zhur. Obschchei Khimii* **19**, 1558 (1949).
139. Brauns, F. E., »The Chemistry of Lignin», Academic Press, New York, 1952, p. 244.
140. Freudenberg, K., Belz, W., and Niemann, C., *Ber.* **62**, 1554 (1929).
141. Abrahamson, B., Lindgren, B. O., and Hägglund, E., *Svensk Papperstidn.* **51**, 471 (1948).
142. Yorston, F. H., and Pichette, A. H., *Pulp Paper Mag. Can.* **50**, no. 12, 114 (1949).
143. Bethge, P. O., and Carlson, O. T., *Anal. Chim. Acta* **15**, 279 (1956).
144. Adler, E., and Gierer, J., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 480.
145. Adler, E., Delin, S., and Lundquist, K., *Acta Chem. Scand.* **13**, 2149 (1960).

146. Adler, E., and Lindgren, B. O., *Svensk Papperstidn.* **55**, 563 (1952).
147. Adler, E., and Yllner, S., *Svensk Papperstidn.* **57**, 78 (1954).
148. Lindgren, B. O., *Acta Chem. Scand.* **12**, 447 (1958).
149. Hägglund, E., »Chemistry of Wood», Academic Press, New York, 1951, p. 264.
150. Brauns, F. E., *J. Am. Chem. Soc.* **61**, 2120 (1939).
151. Felicetta, V. F., and McCarthy, J. L., *J. Am. Chem. Soc.* **79**, 4499 (1957).
152. Hägglund, E., and Johnson, T., *Biochem. Z.* **202**, 439 (1928).
153. Lindgren, B. O., *Svensk Papperstidn.* **61**, no. 18 B, 79 (1958).
154. Friese, H., Högn, V., and Wille, H., *Ber.* **70**, 1072 (1937).
155. Friese, H., and Stoeck, G., *Ber.* **73**, 1135 (1940).
156. Merewether, J. W. T., *Holzforschung* **11**, 65 (1957).
157. Harris, E. E., *Tappi* **36**, 402 (1953).
158. Hägglund, E., »Chemistry of Wood», Academic Press, New York, 1951, pp. 215, 415.
159. Lindgren, B. O., *Acta Chem. Scand.* **5**, 603 (1951).
160. Lindgren, B. O., *Svensk Papperstidn.* **55**, 78 (1952).
161. Leopold, B., *Acta Chem. Scand.* **6**, 64 (1952).
162. Hägglund, E., *Pappers- och Trävarutidskr. Finland* **11**, 64 (1929); **16**, 383 (1934).
163. Kullgren, C., *Svensk Kem. Tidskr.* **44**, 15 (1932).
164. Gardon, J. L., and Mason, S. G., *Can. J. Chem.* **33**, 1477 (1955).
165. Gardon, J. L., and Mason, S. G., *Ind. Eng. Chem. Chem. Eng. Data Ser.* **3**, 115 (1958).
166. Felicetta, V. F., Ahola, A., and McCarthy, J. L., *J. Am. Chem. Soc.* **78**, 1899 (1956).
167. Hägglund, E., *Svensk Papperstidn.* **49**, 191 (1946).
168. Leopold, B., *Acta Chem. Scand.* **6**, 55 (1952).
169. Freudenberg, K., Lautsch, W., and Piazolo, G., *Cellulosechemie* **22**, 97 (1944).
170. Grafe, V., *Monatsh.* **25**, 987 (1904).
171. Tomlinson, G. H., and Hibbert, H., *J. Am. Chem. Soc.* **58**, 348 (1936).
172. Adler, E., and Häggroth, S., *Acta Chem. Scand.* **3**, 86 (1949).
173. Kratzl, K., *Monatsh.* **78**, 173 (1948).
174. Hägglund, E., and Bratt, L. C., *Svensk Papperstidn.* **39**, 347 (1936).
175. Alvfeldt, O., and Hägglund, E., *Svensk Papperstidn.* **40**, 236 (1937).
176. Hägglund, E., and Heiwinkel, H., *Svensk Papperstidn.* **45**, 128 (1942).
177. Lautsch, W., and Piazolo, G., *Cellulosechemie* **22**, 48 (1944).
178. Leopold, B., *Acta Chem. Scand.* **5**, 936 (1951).
179. Adler, E., and Gierer, J., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 468.
180. Freudenberg, K., Meister, M., and Flickinger, E., *Ber.* **70**, 500 (1937).
181. Richtzenhain, H., *Ber.* **72**, 2152 (1939).
182. Erdtman, H., *Svensk Papperstidn.* **46**, 226 (1943).
183. Lindgren, B. O., and Saedén, U., *Acta Chem. Scand.* **6**, 91 (1952).
184. Berg, G. A., and Holmberg, B., *Svensk Kem. Tidskr.* **47**, 257 (1935).
185. Holmberg, B., *Svensk Papperstidn.* Special Issue, **39**, September, 113 (1936).
186. Leger, F., and Hibbert, H., *J. Am. Chem. Soc.* **60**, 565 (1938).
187. Kleinert, T., *Monatsh.* **80**, 582 (1949).
188. Kratzl, K., *Holzforschung* **10**, 161 (1957).
189. Erdtman, H., in Wise, L. E., and Jahn, E. C., »Wood Chemistry», Second Edition, Vol. 2, Reinhold Publishing Corporation, New York, 1952, p. 999.

190. Erdtman, H., Lindgren, B. O., and Pettersson, T., *Acta Chem. Scand.* **4**, 228 (1950).
191. Gierer, J., and Alfredsson, B., through Adler, E., and Gierer, J., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 467.
192. Gierer, J., Alfredsson, B., and Söderberg, S., *Svensk Papperstidn.* **63**, 201 (1960).
193. Mikawa, H., *J. Chem. Soc. Japan Ind. Chem. Sect.* **54**, 651 (1951), through Refs. 160 and 159.
194. Erdtman, H., *Research* **3**, 83 (1950).
195. Tollens, B., and Lindsey, J. B., *Ann.* **267**, 341 (1892).
196. Klason, P., *Arkiv Kemi, Mineral. Geol.* **3**, no. 5, 1 (1908).
197. Melander, K. H. A., »Sulfitavlutens utsaltbara svavelhaltiga ligninsyror», Dissertation, 1919, University of Lund.
198. Klason, P., *Ber.* **53**, 706, 1862, 1864 (1920).
199. Hägglund, E., *Svensk Papperstidn.* **36**, 131 (1933).
200. Klason, P., Schriften des Vereins der Zellstoff- und Papierchemiker, no. 2. Beiträge zur Kenntnis der chemischen Zusammensetzung des Fichtenholzes, Berlin, 1911.
201. Erdtman, H., *Svensk Papperstidn.* **45**, 315 (1942).
202. Erdtman, H., *Svensk Papperstidn.* **45**, 392 (1942).
203. Aulin-Erdtman, G., *Svensk Papperstidn.* **47**, 91 (1944).
204. Brauns, F. E., »The Chemistry of Lignin», Academic Press, New York, 1952, p. 51.
205. Adler, E., and Gierer, J., in Treiber, E., »Die Chemie der Pflanzenzellwand», Springer Verlag, Berlin, Göttingen, Heidelberg, 1957, p. 449.
206. Aulin-Erdtman, G., *Svensk Papperstidn.* **61**, 194 (1958).
207. Aulin-Erdtman, G., *Tappi* **32**, 160 (1949).
208. Freudenberg, K., and Knof, L., *Chem. Ber.* **90**, 2857 (1957).
209. Erdtman, H., in Paech, K., and Tracey, M. V., »Modern Methods of Plant Analysis», Vol. III, Springer Verlag, Berlin, Göttingen, Heidelberg, 1955, p. 428.
210. Vanzetti, B. L., *Monatsh.* **52**, 163 (1929).
211. Hearon, W. M., and MacGregor, W. S., *Chem. Revs* **55**, 957 (1955).
212. Holmberg, B., *Svensk Kem. Tidskr.* **32**, 56 (1920).
213. Holmberg, B., *Ber.* **54**, 2389 (1921).
214. Hintikka, S. V., *Cellulosechemie* **4**, 93 (1923).
215. Lassenius, T., *Pappers- och Trävarutidskr. Finland* **26**, 73 (1944).
216. Industriens Utredningsinstitut, Norrlandsutredningen. »Tillvaratagande och förädling av mindervärdigt virke samt avfalls- och biprodukter i skogsindustrien», Almqvist & Wicksell, Stockholm, 1942, p. 127.
217. Routala, O., and Pohjola, A., *Pappers- och Trävarutidskr. Finland* **16**, 289 (1934).
218. Adler, E., *Svensk Papperstidn.* **50**, 261 (1947).
219. Sundman, J., *Pappers- och Trävarutidskr. Finland* **29**, 52 (1947).
220. Fluka, A. G., Chemische Fabrik Buchs S. G., Switzerland, »Dowex Ionenaustauscher».
221. Bauman, W. C., Wheaton, R. M., and Simpson, D. W., in Nachod, F. C., and Schubert, J., »Ion Exchange Technology», Academic Press, New York, 1956, p. 182.
222. Samuelson, O., in »Niende Nordiska Kemikermøde», Aarhus, 1956, Vol. II, p. 115.
223. Hartler, N., *Acta Chem. Scand.* **11**, 1169 (1957).

224. Shaw, A. C., *Pulp Paper Mag. Can.* **56**, no. 11, 170 (1957).
225. Shaw, A. C., *Can. Pulp Paper Ind.* **10**, no. 11, 49 (1957).
226. Felicetta, V. F., Lung, M., and McCarthy, J. L., *Tappi* **42**, 496 (1959).
227. Regestad, S. O., and Samuelson, O., *Svensk Papperstidn.* **61**, no. 18 B, 145 (1958).
228. Pregl. F., and Roth, H., »Die quantitative organische Mikroanalyse», Vierte Auflage, Verlag von Julius Springer, Berlin, 1935, p. 220.
229. Piper, C. V., and Bernardin, L. J., *Tappi* **41**, 16 (1958).
230. Fischer, F. G., and Dörfel, H., *Z. physiol. Chem. Hoppe-Seyler's* **301**, 224 (1955).
231. Gee, M., and McCready, R. M., *Anal. Chem.* **29**, 257, (1957).
232. Theander, O., *Svensk Kem. Tidskr.* **70**, 393 (1958).
233. Erdtman, H., and Aulin-Erdtman, G., *Svensk Papperstidn.* **47**, 22 (1944).
234. Bellamy, L. J., »The Infra-red Spectra of Complex Molecules», Second Edition, Methuen & Co., London, John Wiley & Sons, New York, 1958, p. 161.
235. Freudenberg, K., Siebert, W., Heimberger, W., and Kraft, R., *Chem. Ber.* **83**, 533 (1950).
236. Spearin, W. E., *J. Org. Chem.* **15**, 984 (1950).
237. Schmeil, O., and Seybold, A., »Lehrbuch der Botanik», Band II, 56. Auflage, Quelle & Meyer, Heidelberg, 1958, p. 89.

THE LAST VOLUMES OF ACTA POLYTECHNICA

Chemistry including Metallurgy Series

(The predecessor of Acta Polytechnica Scandinavica)

Volume 4

Nr 9 SANDFORD, F, and FRANSSON, S: *The Refractoriness of Some Types of Quartz and Quartzite.* II. Acta P 173 (1955), 24 pp, Sw. Kr 5: 00 UDC 666.76:549.514.51

Nr 10 BJÖRKMAN, L, BJÖRKMAN, M, BRESKY, A, ENEBO, L, and RENNERFELT, J: *Experiments on the Culture of Chlorella for Food Purposes.* Acta P 176 (1955), 18 pp, Sw. Kr 4: 00 UDC 663.11:582.2

Nr 11 WRANGLÉN, G: *Dendrites and Growth Layers in the Electrocrystallization of Metals.* Acta P 182 (1955), 42 pp, Sw. Kr 6: 50 UDC 669.017:548.232.4:669.017:548.52

Nr 12 MATTSSON, E: *The Electrode Process in Metal Deposition from Aqueous Solutions.* Acta P 184 (1955), 56 pp, Sw. Kr 6: 50 UDC 541.135:621.357

Nr 13 EDHBORG, A: *Studies on a Water-Soluble Material from the Masonite Process.* Acta P 197 (1956), 87 pp, Sw. Kr 12: 50 UDC 547.454:542.938:542.941
out of print

ACTA POLYTECHNICA SCANDINAVICA

Chemistry including Metallurgy Series

Ch 1 JART, A: *Fat Rancidity. Summaries of Papers Presented at the 2nd Scandinavian Symposium on Fat Rancidity.* (Acta P 242/1958) 72 pp, Sw. Kr 7: 00 UDC 665.112.2

Ch 2 ERÄMETSÄ, O: *Ion Characteristics; a New Way of Assessing the Chemical Properties of Ions.* (Acta P 249/1958) 22 pp, Sw. Kr 7: 00 UDC 541.7

Ch 3 ERÄMETSÄ, O: *On the Decomposition of Potash Felspar.* (Acta P 260/1959) 17 pp, Sw. Kr 7: 00 UDC 553.61:542.92

Ch 4 *Report of the Committee on Slaughtering Methods Appointed by the Danish Academy of Technical Sciences at the Request of the Ministry of Justice.* (Acta P 264/1959) 35 pp, Sw. Kr 7: 00 UDC 637.513.22

Ch 5 MÄKIPIRTTI, S: *On the Sintering of W-Ni-Cu Heavy Metal.* (Acta P 265/1959) 69 pp, Sw. Kr 7: 00 UDC 621.762:669.275.24.3

Ch 6 PAULSEN, A: *Constitution des Quatre Isomeres de Position des Chloro et Aminomethylbenzodioxannes-1,4, Substitues dans le Noyau Benzenique.* (Acta P 270/1960) 94 pp, Sw. Kr 7: 00 UDC 547.84:615.45

Ch 7 DAHLGREN, S-E: *Physico-Chemical Background of Phosphoric Acid Manufacture by Wet Processes.* (Acta P 271/1960) 15 pp, Sw. Kr 7: 00 UDC 661.634

Ch 8 SUNDIUS, N: *Felspar and its Influence on the Reactions in Ceramics during Burning.* (Acta P. 272/1960), 29 pp, Sw. Kr. 7: 00 UDC 666.6.041.9:553.61

Ch 9 DAHL, OLE: *Gamma Irradiation of Vacuum-Packed Sliced Meat Products.* (Acta P. 276/1960), 24 pp, Sw. Kr. 7: 00 UDC 664.91.037:539.166

Ch 10 ENEBO, LENNART AND PEHRSON, STIG O: *Thermophilic Digestion of a Mixture of Domestic Sewage Sludge and Cellulose Materials.* (Acta P. 281/1960) 40 pp, Sw. Kr. 7: 00 UDC 663.1:628.35:676.16

Ch 11 ASCHAN, L. J.: *Studies on the ternary system Cu-Mg-Si.* (Acta P. 285/1960) 46 pp, Sw. Kr. 7: 00 UDC 669.018.669.3.721.782 (084.2)

Ch 12 ERÄMETSÄ, OLAVI and KOLEHMAINEN, ANTTI: *A Modification of the Ion Characteristic to the Glass Theory and the Glasses Coloured with Lanthanons.* (Acta P. 290/1960). 22 pp. Sw. Kr. 7:00 UDC 666.24.01:546.65

Ch 13 ALERTSEN, AASE RYE: *Ageratochromene, a Heterocyclic Compound from the Essential Oil of Ageratum houstonianum Mill.* (Acta P. 293/1961). 66 pp. Sw. Kr. 7:00 UDC 547.81:547.913

Ch 14 SUNNER P, S. and WADSÖ, I: *Measurements of Heat Effects accompanying the wet Carbonization of Peat in the Temperature Range 20 to 220 Degrees C.* (Acta P. 297/1961). 40 pp Sw. Kr. 7:00 UDC 536.662:662.641

Ch 15 ERÄMETSÄ, OLAVI and KARLSSON, KAJ: *The Chrystal Chemistry of some Sodium Polysulphides.* (Acta P. 301/1961) Sw. Kr. 7:00 UDC 546.222.4'33:539.26

Ch 16 RUNOLINNA, URMAS: *Dry Magnetic Separation of Finely Ground Magnetite in a Rotating Magnetic Field.* (Acta P. 303/1961) Sw. Kr. 7:00 UDC 622.778:622.341

Price Sw. Kr. 14.00